Oscillatory bursting of phasically firing rat supraoptic neurones in low-Ca^{2+} medium: Na^+ influx, cytosolic Ca^{2+} and gap junctions

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1. Whole-cell patch recordings were obtained from supraoptic nucleus (SON) neurones in horizontal brain slices of adult male rats. Low-Ca^{2+} or Ca^{2+}-free perifusion medium induced oscillatory bursting activity in all sixty-nine cells displaying both phasic firing and depolarizing after-potentials (DAPs). In fifteen non-phasic cells without DAPs, Ca^{2+}-free medium produced little or no oscillatory bursting.

2. Typical bursts started with rapid membrane depolarization, resulting in a plateau with superimposed action potentials, and ended several hundred milliseconds later in swift repolarization. Prominent bursting was observed at membrane potentials from −50 to −70 mV, with maximum amplitudes of 12·2 ± 0·7 mV (mean ± S.E.M.) around −70 mV. Development of oscillatory bursting was dependent on reduction of $[Ca^{2+}]_o$, with a threshold for the bursting ⩽ 1·2 mM Ca^{2+}.

3. Bursting was abolished by addition of TTX, Co^{2+}, Ni^{2+} or Mg^{2+} into the Ca^{2+}-free medium, or by replacement of external Na^+ with choline or Li^+. Low concentrations of TEA or increased $[K^+]_o$ prolonged burst durations and enlarged oscillation amplitudes.

4. Voltage-clamp techniques were used to examine the persisent Na^+ current (I_{NaP}), and revealed that low $[Ca^{2+}]_o$ shifted the threshold for I_{NaP} activation in a negative direction and enhanced the amplitude of this current. These changes in I_{NaP} were abolished by adding Co^{2+} or Mg^{2+} to Ca^{2+}-free medium.

5. Direct diffusion of BAPTA or heparin into neurones or bath application of ryanodine suppressed bursting. Oscillations were also eliminated by the uncoupling agents heptanol, halothane or acidification.

6. CNQX, APV, bicuculline, CGP35348 ($GABA_B$ receptor antagonist), promethazine, atropine, *d*-tubocurarine and suramin had no obvious effects on oscillatory bursting. Blockers of transient Ca^{2+}, or hyperpolarization-activating cation currents also did not alter bursting activity.

7. These results suggest that intrinsic burst activity in SON neurones perifused with low-Ca^{2+} or Ca^{2+}-free medium involves enhanced Na^+ influx through persistent Na^+ channels, and requires the presence of rapid intracellular Ca^{2+} mobilization that might also explain the selective existence of oscillatory bursting in phasically firing cells. Intercellular communication through gap junctions appears to be important in determining neuronal activity of the neuroendocrine cells in low-Ca^{2+} medium.

Magnocellular neurones in the hypothalamic supraoptic nucleus (SON) are known to participate in regulation of body fluid homeostasis, reproduction and autonomic functions through release of vasopressin and oxytocin from the posterior pituitary and fibre projections to the lateral hypothalamus (for review see Hatton, 1990). When recorded *in vivo* or *in vitro* using conventional electrophysiological methods, these cells are characterized by distinct firing patterns and membrane events. For example, when activated either physiologically or by depolarizing current injection, vasopressinergic neurones in the rat hypothalamus display phasic firing patterns and depolarizing after-potentials (DAPs) following action potentials. In contrast, the vast majority (Armstrong, Smith & Tian, 1994) of oxytocinergic neurones fire continuously and show no DAPs (Andrew & Dudek, 1983;

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Legendre & Poulain, 1992). Ionic mechanisms implicated in phasic firing and DAPs are not well understood. Although many cation conductances may be involved, it is widely accepted that Ca^{2+} plays indispensable roles in generation of these electrophysiological events (Bourque, Brown & Renaud, 1986; Andrew, 1987). Either blockade of Ca^{2+} influx or chelation of intracellular Ca^{2+} can abolish the phasic activity and DAPs in SON cells (Li, Decavel & Hatton, 1995). Little difference in basic membrane properties, or the type and conductance of membrane ion channels has been observed between vasopressinergic and oxytocinergic SON neurones (Cobbett, Legendre & Mason, 1989; Armstrong *et al.* 1994; Fisher & Bourque, 1995).

Depletion of extracellular Ca^{2+} has been shown to induce epileptiform activity in hippocampal and hypothalamic brain slices (Konnerth, Heinemann & Yaari, 1986; Bouskila & Dudek, 1993; Perez-Velazquez, Valiante & Carlen, 1994). The seizure-like activity results from synchronous summation of bursts of action potentials in nearby neuronal populations. However, membrane conductances underlying generation of Ca^{2+} depletion-induced oscillatory bursting remain unknown.

In this study, we report an intrinsic oscillatory burst activity in SON magnocellular neurones that display phasic firing and DAPs, but not in those with continuous firing and without DAPs. This oscillatory bursting is induced by reduction or removal of extracellular Ca^{2+} in the perifusion medium and is mediated by Na^+ influx. It is also dependent on $[Ca^{2+}]_i$ and eliminated by uncoupling agents. These findings are important in understanding both electrophysiological differences between vasopressinergic and oxytocinergic neurones in the SON and ionic mechanisms underlying low-Ca^{2+}-induced epileptiform activity.

METHODS

Adult (50- to 80-day-old) male Sprague–Dawley rats were gently introduced to a small rodent guillotine (Stoelting Co., Wood Dale, IL, USA) and decapitated, as described previously (Hatton, 1982; Li *et al.* 1995). Their brains were removed and cooled in 4 °C oxygenated medium that contained (mM): NaCl, 126; KCl, 5; $NaHPO_4$, 1·3; $CaCl_2$, 2·4; $MgSO_4$, 1·3; $NaHCO_3$, 26; glucose, 10; and 3(*N*-morpholino)propanesulphonic acid buffer, 5 (pH 7·4, 310 mosmol l^{-1}). Horizontal hypothalamic brain slices (250–300 μm thick) were cut using a Vibratome (Oxford Instruments, UK). After incubation at room temperature (23 °C) for 90 min, the slices were transferred to a submerging recording chamber and perifused with warmed medium (36 °C at 1·0–1·5 ml min^{-1}). Nominally Ca^{2+}-free medium was made by omitting $CaCl_2$ or replacing $CaCl_2$ with 2 mM EGTA. As required, choline or Li^+ was used to replace Na^+ in the perifusion medium in order to reduce $[Na^+]_o$.

Patch electrodes (2·5–3·0 μm o.d.; tip, 4–6 MΩ) were pulled from 1·5 mm borosilicate capillary tubing and were filled with the following solution (mM): potassium gluconate, 140; $MgCl_2$, 2; *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid), 10; K_2ATP, 2; and Li_2GTP, 0·4 (pH 7·25). As indicated, BAPTA (1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid), 0·1 mM Ca^{2+}, or heparin was added to the filling solution in some experiments. Through a perfusion hole in the electrode holder (PESW-M15P; Warner Instrument Co., Hamden, CT, USA), one end of a polyethylene tubing (250 μm o.d.) was inserted to a distance of 1–2 mm from the tip of the patch electrode. The other end of this tubing was connected to a 0·5 ml syringe via a three-way stopcock. This device permitted changes of the internal solution and delivery of test agents into the cell during recording. Solution exchange speed (0·1–0·2 ml min^{-1}) was controlled by a motor, and a volume at least 10 times larger than electrode volume (~40 μl) was exchanged. Fluid overflow left the pipette through a suction hole at the back of the electrode holder. Ag–AgCl electrodes were in contact with perifusion medium through a KCl–agar bridge. A liquid junction potential of −7 mV was corrected for in all voltage recordings.

Whole-cell patch clamp recordings were obtained from SON neurones with a microelectrode amplifier using previously described techniques (Li *et al.* 1995). Patch electrodes approached the SON at an angle of 45 deg under the guidance of a ×50 dissecting microscope. The seals between patch electrodes and the cell membrane were obtained by applying gentle suction and verified by measuring electrotonic potential responses produced by hyperpolarizing current pulses (200 ms, −10 pA). When a stable seal (6–10 GΩ) was achieved, further brief suction was applied to break through the membrane. Whole-cell patch recordings were indicated by observation of the negative membrane potential and action potentials. The series resistance was always < 8 MΩ. To achieve an optimal (or maximal) amplitude of oscillations following perifusion with low-Ca^{2+} or Ca^{2+}-free medium, intracellular injection of −20 to −70 pA current was needed to clamp membrane potentials at −60 to −70 mV. For simplicity, the injected current in control conditions was offset to zero in all figures of this paper. Oscillation amplitude was measured from the baseline to the peak of burst plateau. Both burst duration and the interval between two consecutive bursts were obtained at half-amplitude of membrane potential oscillation. For each neurone these values were determined by averaging ten successive measurements. Routinely, 5 ms duration depolarizing current pulses were applied to elicit action potentials at a membrane potential just below spike threshold to determine if DAPs were present. As reported previously (Li *et al.* 1995), ~60% of SON cells encountered showed both phasic firing and DAPs.

To examine a persistent Na^+ current (I_{NaP}), single-electrode voltage clamp was performed in some experiments. Headstage output was continuously monitored on a separate oscilloscope to ensure adequate settling time. Switching rate was usually set at 7–8 kHz; feedback gain, 1·5–5·0 nA mV^{-1} and low-pass filter, 10 kHz. I_{NaP} was activated by two kinds of depolarizing protocols, i.e. voltage steps and ramps (Alzheimer, Schwindt & Crill, 1993). Step commands of 0·5 s duration clamped membrane potentials from holding potentials (−85 to −70 mV) to more positive levels. Voltage ramps at rates of 1 mV s^{-1} and/or 45 mV s^{-1} were run and, in five cells where both types of ramps were tested, the only difference in yielded current traces was that the slower ramps evoked I_{NaP} without activation of a fast Na^+ current (I_{NaF}). Three to eight consecutive current traces were filtered, averaged and then plotted against voltage. The change in I_{NaP} was determined by comparing ion currents obtained before and during perifusion of slices with low-Ca^{2+} medium or test agents. To avoid activating the Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$), depolarization of the voltage step or ramp was always below the activation threshold of $I_{K(Ca)}$ (−30 to −20 mV). Due to space clamp problems that always exist in experiments on slice preparations, the I_{NaF} responsible for fast depolarization of action potentials could not be measured

accurately, and thus these data were not used for further analysis. All data acquisition and analyses were performed using an IBM-compatible computer operating AXOTAPE and pCLAMP (Axon Instruments).

2-Amino-5-phosphonovaleric acid (APV), 4-aminopyridine (4-AP), atropine sulphate, bicuculline methiodide, $CoCl_2$, halothane, 1-heptanol, $NiCl_2$, promethazine, tetrodotoxin (TTX) and *d*-tubocurarine chloride were obtained from Sigma. CGP35348, the selective $GABA_B$ receptor antagonist, was a gift from Ciba-Geigy Ltd (Basel, Switzerland). Caesium chloride (Fisher, Fair Lawn, NJ, USA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; RBI, Natick, MA, USA), ryanodine, suramin (both from Calbiochem, La Jolla, CA, USA) and tetraethylammonium chloride (TEA; Aldrich) were also used in these experiments. Both heptanol and halothane were dissolved in dimethyl sulphoxide (DMSO) as stock. In perifusion medium, DMSO concentration was less than 0·1% (v/v), and had no detectable effect on recorded neuronal activity.

RESULTS

Whole-cell patch recordings in this study were obtained from 132 SON neurones, with resting membrane potentials of $-59{\cdot}2 \pm 0{\cdot}6$ mV (mean ± S.E.M.), action potentials of $88{\cdot}3 \pm 0{\cdot}7$ mV and input membrane resistances of $405{\cdot}4 \pm 11{\cdot}3$ MΩ.

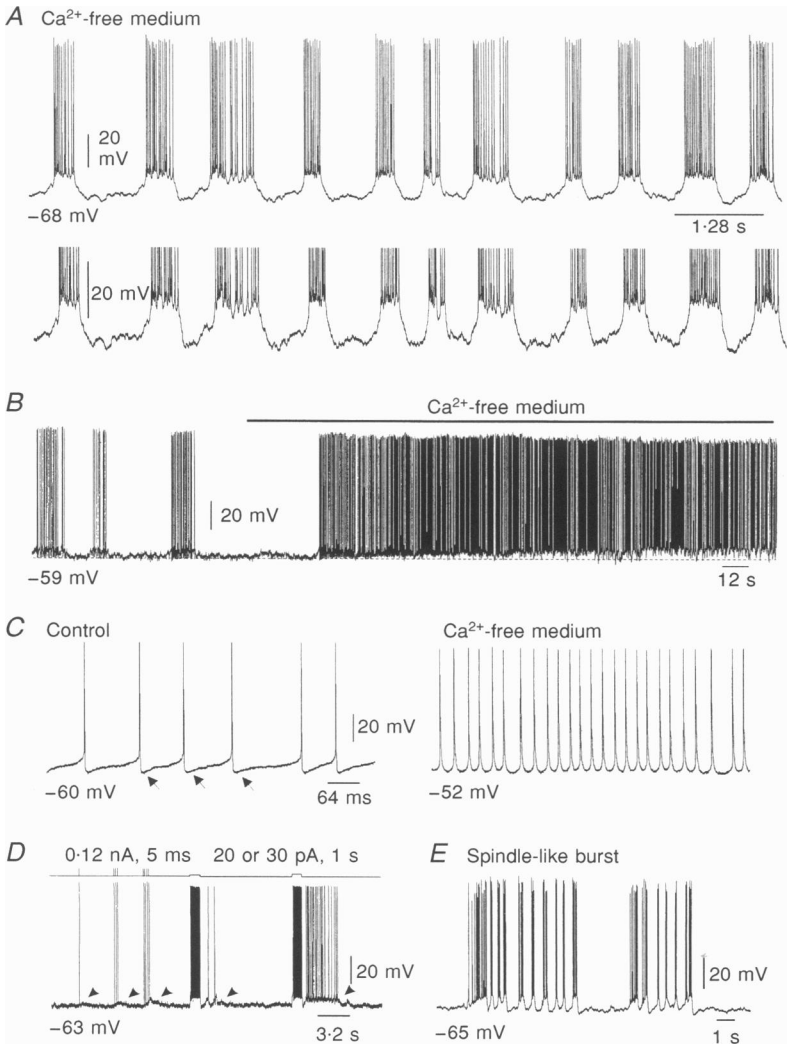

Figure 1. Responses of SON neurones exhibiting both phasic firing and depolarizing after-potentials (DAPs) to extracellular Ca^{2+} depletion

A, whole-cell voltage records obtained following perifusion of Ca^{2+}-free medium for 7 min. Membrane oscillations with trimmed action potentials at higher gain are shown in the lower trace. In this neurone, 40 pA negative DC current was injected to clamp interburst membrane potential close to −68 mV. *B*, Ca^{2+}-free medium induced membrane depolarization and increased firing rate. In this and the following figures, filled bars above records indicate the time during which test medium or agents were bath applied. Note a phasic firing pattern in control medium (2·4 mM Ca^{2+}). *C*, after-hyperpolarizations (AHPs, arrows) were also abolished in Ca^{2+}-free medium. *D*, DAPs (arrowheads) were observed during perifusion of Ca^{2+}-containing medium. Upper trace represents current record, and lower trace voltage record. *E*, voltage trace showing spindle-like bursting found in a few cells. In this figure, all whole-cell records except *E* were obtained from the same SON neurone.

General characteristics of bursting

When Ca^{2+}-free medium with or without 2 mM EGTA replaced control perifusion medium containing 2·4 mM Ca^{2+}, oscillatory burst activity was observed in sixty-nine of sixty-nine SON neurones displaying phasic firing and DAPs (Fig. 1*A* and *D*). Bursting always started within 6 min, but often within 2 min after the onset of perifusion of the Ca^{2+}-free medium, and was accompanied by 3–10 mV membrane depolarization, reduced after-hyperpolarizations (AHPs), lowered spike threshold and increased firing rate (Fig. 1*B* and *C*), probably due to a reduction in $I_{K(Ca)}$ and changes in Na^+ channel gating and conductance (see Na^+ influx; Moczydlowski & Schild, 1994). A typical burst (Fig. 1*A*) started with rapid membrane depolarization, which then resulted in a plateau with superimposed action potentials. The burst ended with swift repolarization, often followed by slow hyperpolarization. In 94% (65) of these sixty-nine cells recorded, bursts were observed to repeat with relatively regular intervals (453·0 ± 20·1 ms, $n = 65$) for hours (maximum observed was 3 h) or until the membrane–electrode seal was broken. In four cells, consecutive bursts were followed by periods of silence (Fig. 1*E*), a pattern which is similar to spindle-like oscillations found in cat thalamocortical neurones (Leresche, Lightowler, Soltesz, Jassik-Gerschenfeld & Crunelli, 1991).

The observed bursting is not a result of dilution or leakage of intracellular elements following the whole-cell patch recordings. Immediately following membrane rupture the burst activity was revealed in SON cells pre-perifused with Ca^{2+}-free medium. When Ca^{2+}-containing medium was re-introduced, neurones were hyperpolarized and the bursting disappeared ($n = 9$).

Dependence on $[Ca^{2+}]_o$ and membrane potential

The development and degree of oscillatory bursting were dependent on lowering external Ca^{2+} concentration (Fig. 2*A*). When the Ca^{2+} concentration in the medium was between 0·8 and 1·2 mM, burst firing began to be observed ($n = 7$), suggesting that a threshold for the bursting is around 1 mM. Further reduction of Ca^{2+} concentration induced larger oscillations and higher burst frequency. When Ca^{2+}-free medium containing 2 mM EGTA was perifused, the bursting was usually seen sooner (within 3 min) than in the Ca^{2+}-free medium without EGTA, but amplitudes were still in similar voltage ranges ($n = 9$).

Prominent oscillatory bursts were seen when membrane potentials were −50 to −70 mV. At more depolarized voltage levels, cells fired continuously. As membrane potentials became more negative than −50 mV, amplitudes of membrane potential oscillation increased while durations

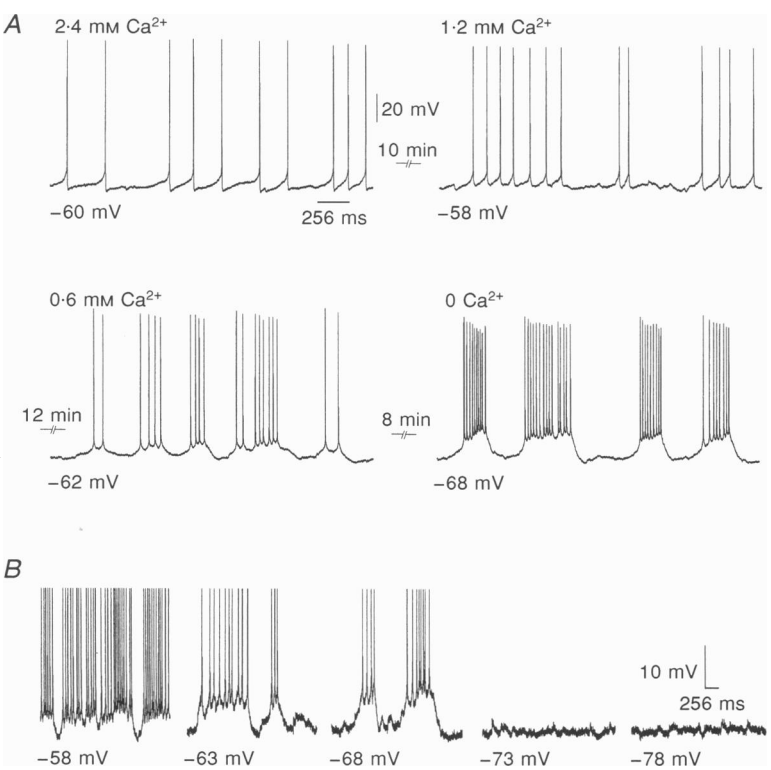

Figure 2. $[Ca^{2+}]_o$ and voltage dependence of oscillatory bursting

A, relationship between bursting and $[Ca^{2+}]_o$ for 1 cell. Note that the maximal oscillation amplitudes obtained are presented. *B*, manipulation of membrane potential changed both oscillation amplitude and the frequency of bursting. Enlarged voltage traces obtained at different membrane potentials from another cell are presented. Hyperpolarization negative to −70 mV virtually cancelled all burst activity.

were reduced (Fig. 2*B*). Maximum amplitudes were always seen around −70 mV with absolute values of 5–25 mV (12·2 ± 0·7 mV, $n = 69$). Burst duration was more variable than its amplitude and, in a given cell, the longest burst could be 10 times as long as the shortest. At membrane potentials around −70 mV, the burst durations in sixty-nine SON cells recorded ranged from 50 to 1200 ms (295·3 ± 24·2 ms). When cells were further hyperpolarized, no bursting was observed (Fig. 2*B*). The generation of bursts associated with membrane events comparable to fast prepotentials (FPPs), proposed to be gap junction-related electrophysiological events (Perez-Velazquez *et al.* 1994), was observed in four cells with membrane potentials of −60 to −70 mV.

Difference in various cell populations

Consistent with the previous observations (Andrew, 1987; Li *et al.* 1995), ~60% of SON neurones encountered displayed phasic firing and DAPs, and the remaining cells (only 15 of which were studied) fired continuously and had neither spontaneously occurring nor current-evoked DAPs. SON neurones with different firing patterns were perifused with Ca^{2+}-free medium and all cells displaying both phasic firing patterns and DAPs demonstrated oscillatory bursting (Fig. 1*B* and *D*, $n = 69$). In all fifteen recorded SON cells showing continuous firing, however, little or no bursting was observed during perifusion of Ca^{2+}-free medium for 20–30 min, although depolarization, reduced AHPs and lowered spike thresholds occurred (Fig. 3). The mean oscillation amplitude of these fifteen cells was significantly smaller than that of the phasic neurones (2·1 ± 0·7 *vs.* 12·2 ± 0·7 mV, ANOVA, $P < 0{\cdot}001$). This finding suggests that neuronal activities induced by low-Ca^{2+} medium are specific among SON cells and that cellular mechanisms underlying the bursting might also be involved in generation of DAPs and phasic firing in control medium.

Na^+ influx

$[Ca^{2+}]_o$ is known to suppress Na^+ influx through screening negative charges on the membrane surface and blockade of Na^+ channels (for review see Moczydlowski & Schild, 1994). A recent observation shows that reduced inactivation of Na^+ currents by ionophores induces a regenerative activity in SON neurones in a Ca^{2+}-free high-Mg^{2+} medium (Inenaga, Nagatomo, Kannan & Yamashita, 1993). To ascertain if Na^+ influx was implicated in initiating the burst activity observed in this study, we perifused SON neurones with Ca^{2+}-free medium containing a Na^+ channel blocker, TTX, during oscillatory bursting. In all eight SON cells tested (i.e. $n = 8/8$), 1–2 μM TTX completely abolished the bursting

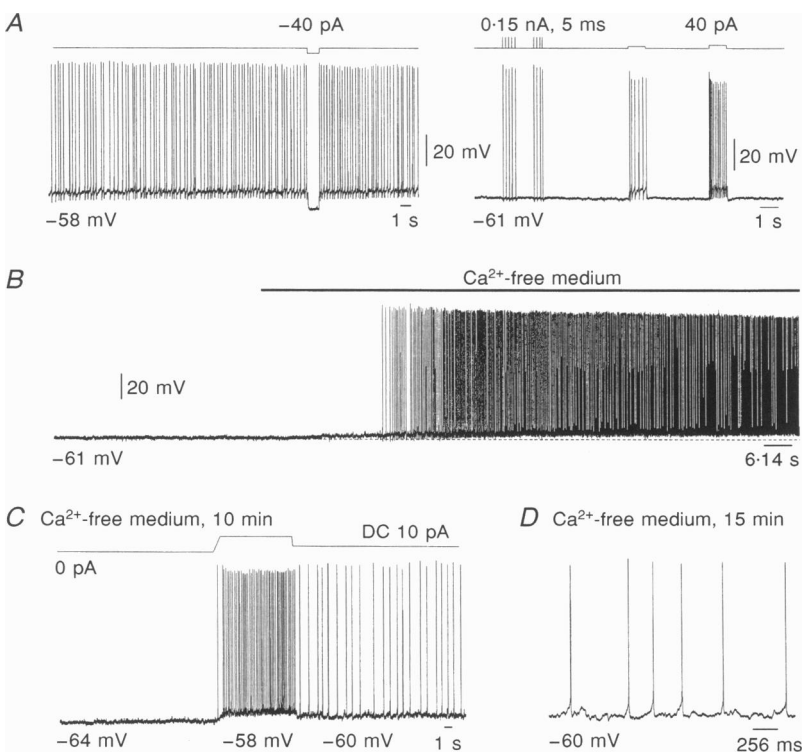

Figure 3. Depletion of external Ca^{2+} induced no burst activity in SON cells with continuous firing patterns

A, voltage records obtained from a SON cell showing regular neuronal activity (left) and no DAPs (right) during perifusion of Ca^{2+}-containing medium. *B*, removal of external Ca^{2+} produced depolarization with increased firing rate in the same cell. However, manipulation of membrane potential (*C*) and prolonged perifusion of Ca^{2+}-free medium (*D*) failed to reveal burst activity.

and produced 2–5 mV membrane hyperpolarization (Fig. 4*A*). When the Ca^{2+}-free medium, in which membrane-impermeable choline replaced 82% of the Na^+, was perifused, bursting was eliminated in all six cells tested (Fig. 4*B*). These effects were always accompanied by membrane depolarization and increased input membrane resistance. Li^+ is known to pass through Na^+ channels but is poorly extruded from inside the cell and elicits Na^+-dependent processes. Therefore, we also reduced $[Na^+]_o$ by replacing 82% of the Na^+ in the medium with Li^+ in another six cells. Such treatment was found to abolish or reduce the bursting in all of these cells. Similarly, membrane depolarization was also seen in each of these cases. These effects appeared within 1–2 min and lasted more than 30 min. When 2 mM Co^{2+} ($n = 5$), 1–5 mM Ni^{2+} ($n = 9$) or 10 mM Mg^{2+} ($n = 6$) was added to the Ca^{2+}-free medium, elimination of bursting was observed in all twenty cells tested (Fig. 5), but burst recovery was much faster in these cases (5–10 min) than in the TTX-treated slices. A lower concentration (4 mM) of Mg^{2+} did not affect bursting ($n = 2/2$). These results suggest that enhanced Na^+ influx is essential for generating the oscillatory bursting in SON neurones perifused with low-Ca^{2+} medium. This effect is most probably due to reduced influences of divalent cations on Na^+ channel gating and single-channel conductance. Sustained depolarizations observed in the choline- or Li^+-containing medium indicate that, during bursts, elevated $[Na^+]_i$ due to Na^+ influx can also trigger processes which repolarize SON neurones.

Persistent Na^+ current (I_{NaP})

Since membrane oscillation depends on $[Na^+]_o$ and has a rise time of tens of milliseconds, voltage-clamp experiments were performed to investigate the change in I_{NaP} following

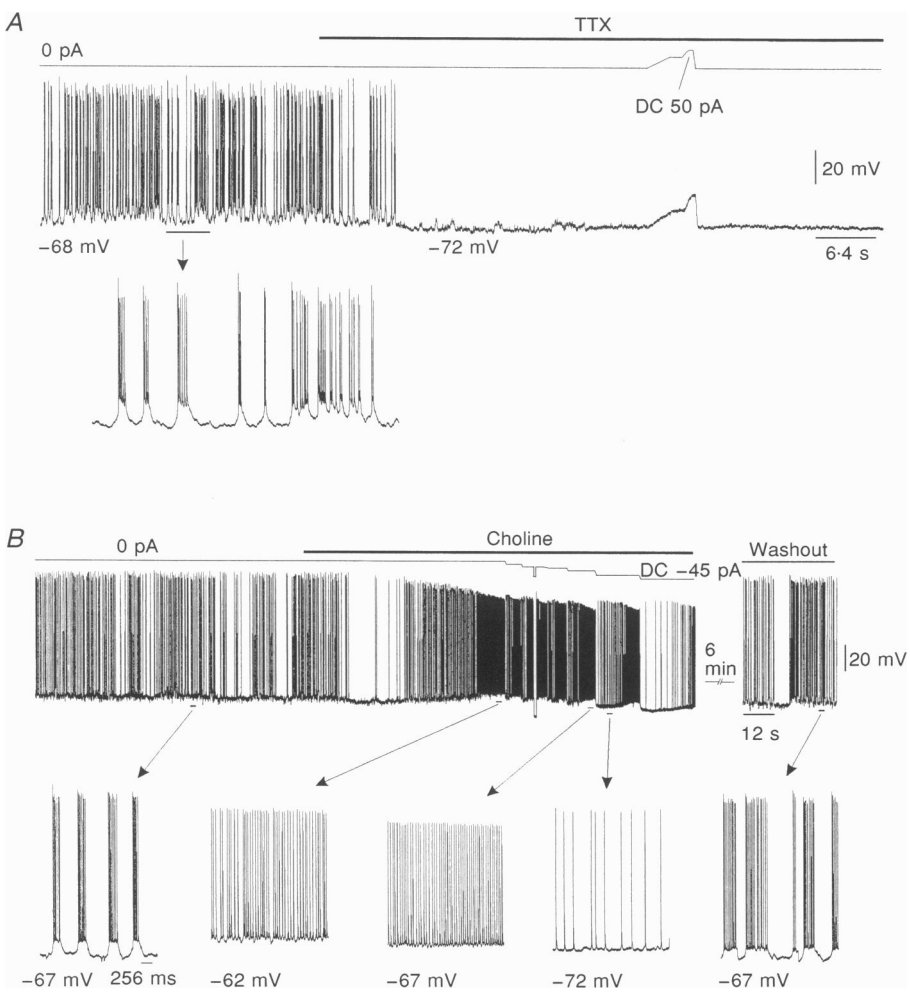

Figure 4. Dependence of bursting on $[Na^+]_o$

A, TTX (2 μM) application (bar) abolished Ca^{2+} depletion-induced bursting and caused membrane hyperpolarization. *B*, replacement of 82% external Na^+ with choline reversibly suppressed bursting and produced membrane depolarization. In this and the following figures, arrows are used to show the time when expanded voltage traces were obtained, and washout is with Ca^{2+}-free medium. Since the oscillatory bursting is voltage dependent, manipulation of membrane potential by injecting current through electrodes during experiments, as shown in this and the following figures, was always performed to investigate the effects of test agents or media.

depletion of external Ca^{2+}. Voltage steps clamping membrane potentials from holding levels (−85 to −70 mV) to −55 mV or more positive levels usually activated an inward current (Fig. 6*A* and *B*). This current was completely blocked by external application of TTX and, in contrast to the I_{NaF} responsible for action potentials, displayed little inactivation during depolarizing steps of hundreds of milliseconds ($n = 8/8$). Its peak amplitude ranged from −50 to −300 pA and was reduced by a leakage current and, with large voltage steps, outward K^+ currents. When voltage ramps at rates of 1 mV s^{-1} and/or 45 mV s^{-1} were used, we observed a TTX-sensitive inward current with a low activation threshold (−56·7 ± 2·2 mV, $n = 22$) and a slow time course, both of which differed from I_{NaF} (Fig. 6*C*). This current, therefore, was designated as I_{NaP} due to its similarity to the analogous current described in other cell preparations (Jahnsen & Llinás, 1984; Stafstrom, Schwindt, Chubb & Crill, 1985).

Perifusion of slices with Ca^{2+}-free medium enhanced I_{NaP} and shifted the activation threshold in a negative direction (Fig. 6*A* and *B*, $n = 8/8$). This effect was not due to removal of $I_{K(Ca)}$ because little $I_{K(Ca)}$ was activated by the test protocols used (see Methods) and also because similar changes in I_{NaP} were observed even after blockade of $I_{K(Ca)}$ by either external application of 10–20 mM TEA and 5–10 mM 4-AP (Fig. 6, $n = 8$) or internal diffusion of 11 mM BAPTA (Fig. 7*A*, $n = 5$; Li *et al.* 1995). Consistent with the results obtained using step commands, ramp voltage protocols revealed that Ca^{2+}-free medium shifted I_{NaP} activation threshold by 13·4 ± 1·5 mV ($n = 23$). Similar changes in I_{NaP} were also observed in cells showing no DAPs ($n = 5$). Since divalent ions have been shown to cancel the oscillatory bursting, whether they influence I_{NaP} was examined in the present experiments. We found that addition of 2 mM Co^{2+} ($n = 4$) or 10 mM Mg^{2+} ($n = 3$) into Ca^{2+}-free medium abolished the negative shift of the I_{NaP}

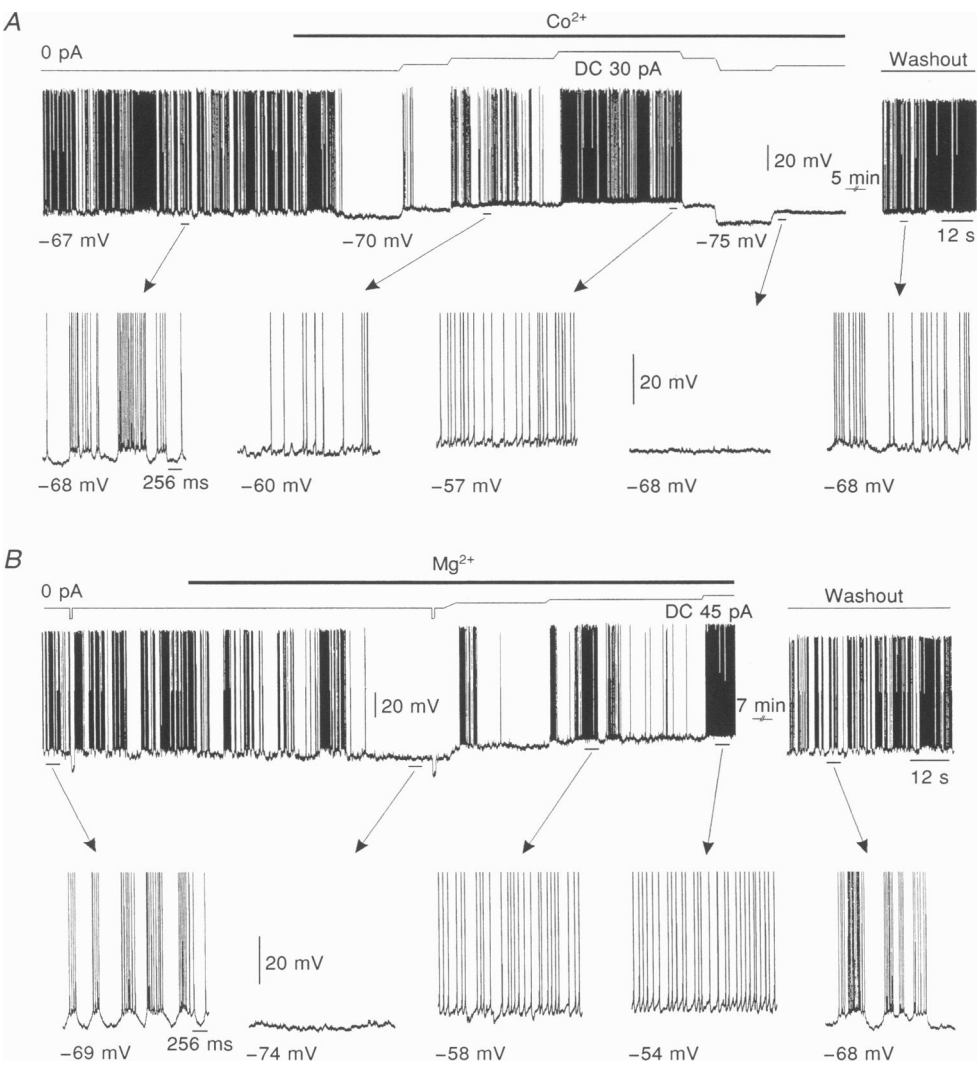

Figure 5. Inhibitory effects of divalent cations on bursting

Co^{2+} (2 mM; *A*) and Mg^{2+} (10 mM; *B*) reversibly inhibited bursting and induced membrane hyperpolarization. In both *A* and *B*, expanded voltage traces (lower traces) were obtained at the different times shown in the continuous records (upper traces) to illustrate changes in burst activity.

activation curve in all seven cells tested (Fig. 7*B*). These results suggest that I_{NaP} is important in the bursting induced by low $[Ca^{2+}]_o$. The almost complete absence of bursting observed in SON cells without DAPs is, therefore, unlikely to result from differences in I_{NaP}.

Involvement of K^+ current

Ca^{2+}-dependent, as well as other, K^+ currents have been proposed to control burst termination in many cell preparations (Alger & Nicoll, 1980). It is known that burst activities can be facilitated by application of K^+ channel blockers (Schwindt, Spain, Foehring, Stafstrom, Chubb & Crill, 1988; Christie, Williams & North, 1989). In the present study, therefore, we examined K^+ current modulation of oscillatory bursting induced by Ca^{2+}-free medium. As shown in Fig. 8*A*, Ca^{2+}-free medium containing 1–2 mM TEA prolonged burst durations and enhanced oscillation amplitudes. This was seen in six of six cells tested. In two SON cells without oscillatory bursting, however, TEA-

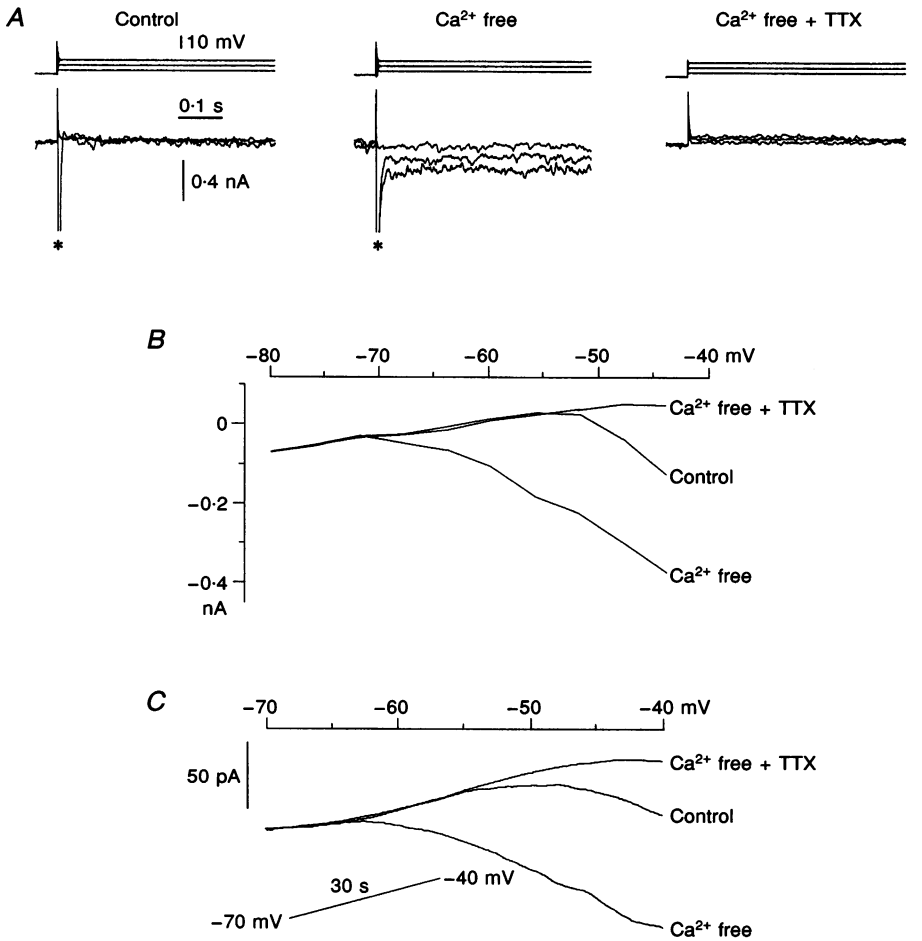

Figure 6. Enhancement of I_{NaP} following removal of external Ca^{2+}

All data shown in this figure were obtained from a SON neurone using an electrode containing a filling solution without BAPTA. *A*, current traces evoked by depolarizing commands clamping the membrane potential from −70 mV (holding potential) to −64, −56 and −48 mV. Perifusion of slices with Ca^{2+}-free medium for 8 min induced an inward current with little inactivation (centre), which was quite small and required larger voltage steps to activate in control (Ca^{2+}-containing) medium (left). TTX (1 μM), added to Ca^{2+}-free medium, blocked I_{NaP} within 3 min (right). Asterisks indicate the I_{NaF} characterized by rapid inactivation. Upper traces show recorded membrane potentials during voltage clamps, and overshoots at the onset of voltage steps largely represent an inability to clamp neuronal action potentials properly in slice preparations. Throughout this experiment, TEA (20 mM) and 4-AP (10 mM) were added to perifusion media, and AHP elimination and prolonged spike repolarization were observed before perifusion of Ca^{2+}-free medium. *B*, *I–V* curve demonstrating that depletion of external Ca^{2+} enhances I_{NaP} and shifts its activation threshold toward the hyperpolarizing direction. Measurements were made at the end of 500 ms voltage steps. *C*, currents evoked by slow (1 mV s^{-1}) voltage ramps during consecutive perifusion of the same neurone with control, Ca^{2+}-free, and TTX-containing Ca^{2+}-free media. The slow ramp protocol, evoking I_{NaP} without concomitant activation of I_{NaF}, reveals changes in I_{NaP} similar to that observed when step commmands were applied (*B*). Averages of three current traces are shown. The inset (below) is the ramp protocol used.

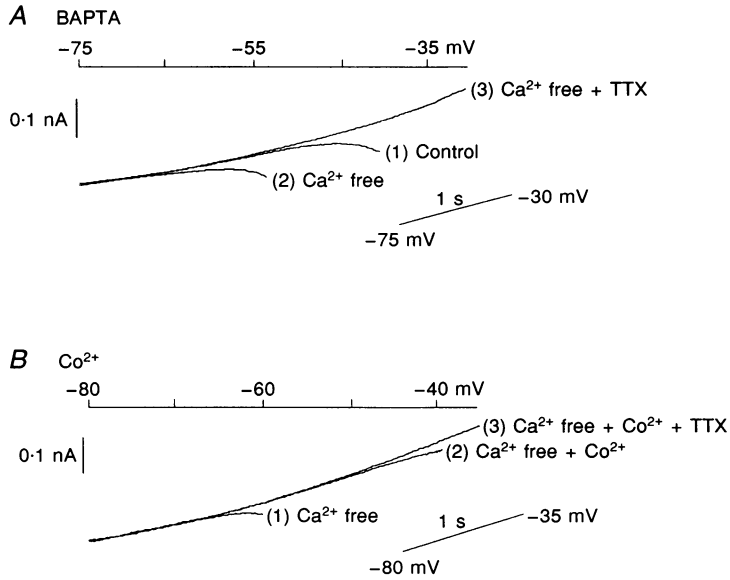

Figure 7. Alteration in I_{NaP} activation threshold

A, current traces obtained from a cell using an electrode filled with a solution containing 11 mM BAPTA. The inset (below) illustrates the ramp voltage protocol used. Control curve (1) was obtained after blockade of Ca^{2+}-dependent AHP and more than 20 min in Ca^{2+}-containing medium. Perifusions with Ca^{2+}-free medium for 10 min (2) resulted in a negative shift of the I_{NaP} activation curve, an effect which was later cancelled by 2 μM TTX (3). *B*, the influence of Co^{2+} on I_{NaP} activation. Current traces were acquired following perifusion of the slice with media containing zero Ca^{2+} for 10 min (1), zero Ca^{2+} plus 2 mM Co^{2+} for 10 min (2), and zero Ca^{2+} plus 2 mM Co^{2+} plus 2 μM TTX for 3 min (3). Co^{2+} shifted the I_{NaP} activation curve in a positive direction. In this figure, averages of 8 consecutive current traces are shown. Some segments of current traces were cut because I_{NaF} was activated and, thus, assessment of potential changes in I_{NaP} amplitude was not available.

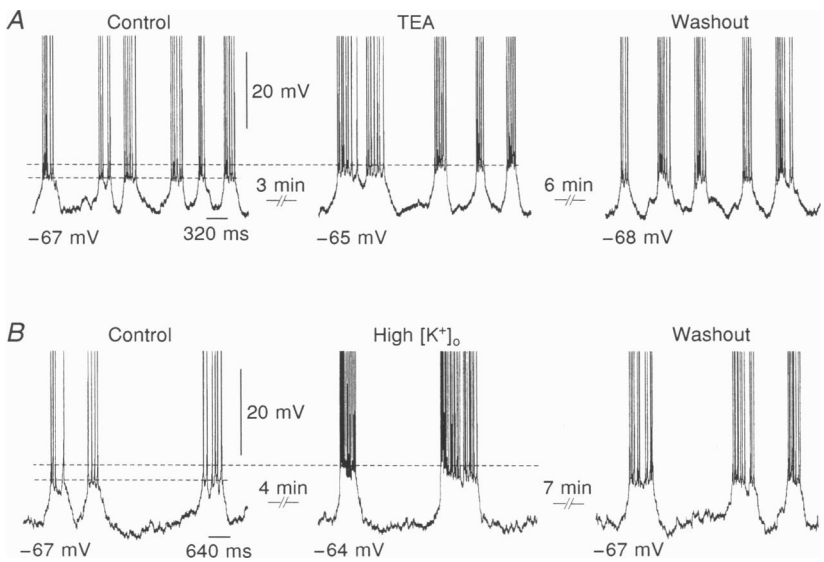

Figure 8. TEA and high $[K^+]_o$ enhanced oscillation amplitude of SON cells

A and *B*, voltage traces obtained before (left), following perifusion of 2 mM TEA for 2 min (*A*, centre) or 10 mM K^+ (*B*, centre) for 3 min, and 3·5 min (*A*, right) or 4·5 min (*B*, right) after the end of agent applications. Dashed lines are used to show the differences in oscillation amplitude. Since 10–20 mM K^+_o, but not 1–2 mM TEA, always induced membrane depolarization, increased (negative) current injection was needed during high-$[K^+]$ application to keep membrane potential close to that of control for comparison (*B*, centre). For simplicity, only 1 expanded record during test agent application is shown in *A* and *B*, although similar procedures to those demonstrated in Figs 4 and 5 were used.

containing Ca^{2+}-free medium failed to induce any burst activity, although depolarization was seen in one of these cells. A higher concentration of TEA (10 mM) first prolonged bursting, then induced a shoulder on the falling phase of the action potential, which masked burst activity ($n = 2/2$). When raising $[K^+]_o$ in the Ca^{2+}-free medium from 5 to 10–20 mM, we saw a trend towards enlarged oscillations and prolonged bursts (Fig. 8*B*, $n = 4/4$) before 30–40 mV depolarizations abolished all spikes and bursting. During washout, those enlarged oscillations and prolonged bursts appeared again before full recovery. These results suggest that K^+ outward currents participate in termination or repolarization of the bursting.

Dependence on intracellular Ca^{2+}

Endogenous Ca^{2+} sequestration mechanisms have been implicated in regulation of neuronal activities in CNS neurones (Baimbridge, Celio & Rogers, 1992). SON neurones with DAPs and phasic firing (i.e. vasopressinergic neurones) tend to immunostain weakly for calbindin-D_{28k}, a member of the Ca^{2+}-binding protein family (Li *et al.* 1995). Since bursting was always observed in SON cells with DAPs and phasic firing but not in cells with continuous firing, we examined the dependence of oscillatory bursting on $[Ca^{2+}]_i$.

When patch electrodes filled with a solution containing 11 mM BAPTA were used, little or no bursting was observed during perifusion with Ca^{2+}-free medium for 20–30 min in all seven SON cells tested (Fig. 9*A*). Mean oscillation amplitude was $1{\cdot}1 \pm 0{\cdot}8$ mV, which was significantly different from that of phasic neurones ($12{\cdot}2 \pm 0{\cdot}7$ mV, ANOVA, $P < 0{\cdot}001$). As shown in our previous study (Li *et al.* 1995), BAPTA at this concentration cancelled both DAPs and phasic firing within 1 min following whole-cell recording and therefore it was not possible to identify these cells according to their firing patterns. Since approximately 40% of SON cells recorded were those firing continuously and lacking DAPs in Ca^{2+}-containing medium, it is possible

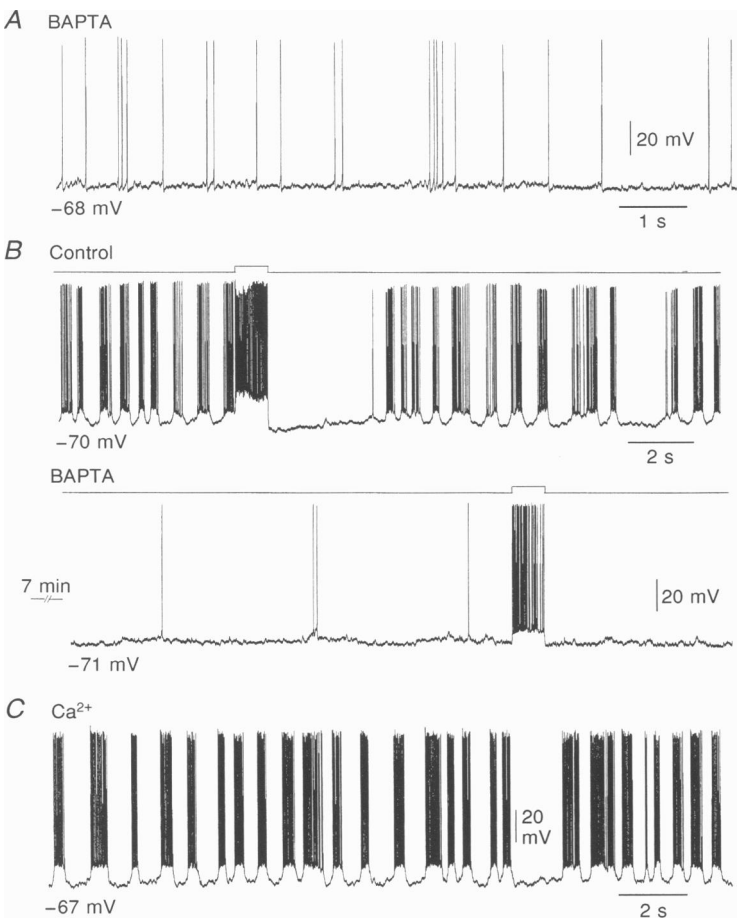

Figure 9. Influence of $[Ca^{2+}]_i$ on bursting

A, voltage record obtained from a SON cell using a patch electrode filled with 11 mM BAPTA-containing solution, showing no bursts following perifusion of Ca^{2+}-free medium with 2 mM EGTA for 15 min. *B*, records obtained from another cell in Ca^{2+}-free EGTA-containing medium demonstrate burst activity (upper trace), which was abolished 7 min after exchange of control internal solution with 11 mM BAPTA-containing solution (lower trace). BAPTA diffusion into the cell was also indicated by cancelling AHPs. Current pulses used were 40 pA, 1 s. *C*, voltage trace obtained 7 min following perifusion of Ca^{2+}-free EGTA-containing medium using a patch electrode filled with a solution containing 0·1 mM Ca^{2+}.

that in some of those seven cells the failure to find bursting was not due to BAPTA. To obtain further evidence supporting Ca^{2+} dependence, the electrode-filling solution in some experiments was exchanged for 11 mM BAPTA-containing solution after bursting had been observed in the Ca^{2+}-free medium. In all three cells tested, intracellular perfusion with BAPTA abolished both bursting and Ca^{2+}-dependent AHPs (Fig. 9*B*). In contrast, prominent bursting was observed in all eight SON neurones when we used patch electrodes filled with a solution containing 0·1 mM Ca^{2+} (Fig. 9*C*), which has been shown to reveal DAPs and phasic firing in SON cells (Li *et al.* 1995).

In five cells recorded using electrodes containing 11 mM BAPTA, voltage ramps were applied to examine I_{NaP}. We found that in all these cells, Ca^{2+}-free medium also induced a negative shift of the activation curve, similar in extent to that shown in experiments using BAPTA-free electrodes (Fig. 7*A*). These results suggest that BAPTA, and endogenous buffers, abolish the bursting via controlling $[Ca^{2+}]_i$ but do not directly influence Na^+ conductances.

Recently, endogenous mechanisms such as internal Ca^{2+} stores have been proposed to participate in regulation of $[Ca^{2+}]_i$ in CNS neurones (Lipscombe, Madison, Poenie, Reuter, Tsien & Tsien, 1988) and neuronal activity (Sah & McLachlan, 1991). Thus, blockers of ryanodine and inositol 1,4,5-trisphosphate (IP_3) receptors were used to examine possible roles of Ca^{2+} released from internal stores in burst generation. In six of six SON cells with DAPs and phasic firing, little or no bursting was observed during perifusion of the Ca^{2+}-free medium for 15 min either with 10 μM ryanodine, or following pretreatment with 10 μM ryanodine for 10–15 min (Fig. 10). Addition of ryanodine into the Ca^{2+}-free medium after bursting was established also reduced oscillation amplitude ($n = 3$). Electrodes filled with a solution containing 4 mg ml^{-1} heparin were used for recording in some experiments. Ca^{2+}-free medium was perifused at least 5 min after membrane rupture to allow a sufficient amount of heparin to diffuse into the SON cells. Depletion of external Ca^{2+} for 20–30 min failed to induce oscillatory bursting in all nine cells with DAPs and phasic firing. These results further implicate intracellular Ca^{2+} mobilization as being important in generating oscillatory bursting in low-Ca^{2+} or Ca^{2+}-free medium.

Effects of common uncoupling agents

Evidence for neuronal coupling via gap junctions exists for many areas of the CNS, including the SON (for review see Hatton, 1990). Such coupling has been proposed to co-ordinate neuronal activity and participate in epileptogenesis (Llinás & Yarom, 1986; Christie *et al.* 1989). A recent study shows that Ca^{2+}-free medium increases dye-coupling incidence between neurones and produces seizure-like activity in the hippocampus, effects which can be abolished by uncoupling agents (Perez-Velazquez *et al.* 1994). To determine if gap junctional conductances function in the establishment of Ca^{2+}-free-induced bursting, conventional uncoupling agents (Bennett, Barrio, Bargiello, Spray, Hertzberg & Saez, 1991) were applied in the present experiments.

Perifusion of Ca^{2+}-free medium containing either halothane (1·25–5·0 mM, $n = 4$) or heptanol (1·7–3·0 mM, $n = 7$), or acidified (pH 5·9–6·0, $n = 2$) Ca^{2+}-free medium abolished or reduced the bursting in all thirteen cells tested (Fig. 11).

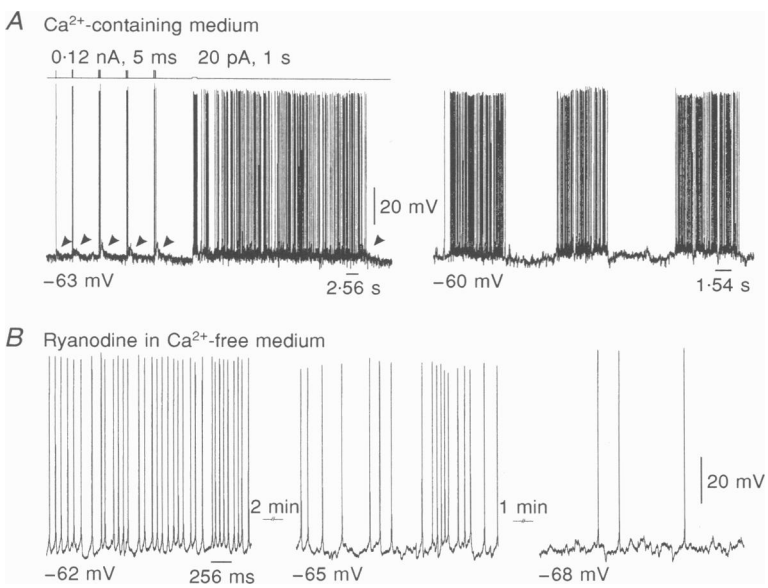

Figure 10. Blockade of intracellular ryanodine receptors prevented SON neurones from bursting

A, records obtained from one cell in Ca^{2+}-containing medium showing DAPs (left, arrowheads) and phasic firing (right). *B*, Ca^{2+}-free medium containing 10 μM ryanodine for 10 min produced no bursting activity, regardless of manipulation of the membrane potential.

These blocking effects appeared within 2 min of the start of perifusion and complete recovery of the bursting was seen 10–20 min after the end of perifusion. Although all test agents induced membrane depolarization with inconsistent change in membrane input resistance, heptanol was distinguished from halothane or acidification by its effects of arresting action potentials. Reduced heptanol concentration (0·4–0·8 mM, $n = 3$) had little influence on the bursting. These results suggest that gap junctions may be involved in generation of the bursting and these common uncoupling agents can block both the bursting and seizure-like activities, probably both by gating gap junctional conductances and by inhibiting Na^+ influx (Elliott, Elliott, Harper & Winpenny, 1992).

Influence of chemical synaptic inputs

Ca^{2+} influx is usually required for neurotransmitter release from presynaptic terminals. Although it has been shown in previous studies that fast synaptic transmission is markedly reduced or abolished in low-Ca^{2+} or Ca^{2+}-free medium (Hatton, 1982), possible influences of synaptic inputs on bursting were evaluated.

To determine if oscillatory bursting in Ca^{2+}-free medium can be evoked by fast synaptic events such as EPSPs, repeated short current pulses (5 ms) at an intensity enough to trigger spikes were used to simulate effects of fast depolarization. The results showed that short depolarizations themselves were not able to initiate bursting reliably, but could favour or speed up the depolarizing phase without a significant change in burst interval ($n = 11$). Prolonged depolarizations evoked by multiple current pulses also failed to trigger bursting ($n = 8$).

Anatomical and physiological studies have demonstrated that the SON receives abundant afferent fibres and that glutamate, GABA, histamine, ACh and ATP released from nerve terminals can produce fast postsynaptic potentials in SON magnocellular cells (Hatton, 1990; Day, Sibbald & Khanna, 1993). Using respective postsynaptic receptor blockers, we investigated the possibility that these substances generate bursting. The results showed that bath application of CNQX (10–20 μM, $n = 5$), APV (20–50 μM, $n = 5$), bicuculline (10 μM, $n = 5$), the $GABA_B$ receptor blocker CGP35348 (0·1 mM, $n = 3$), the histamine H_1 receptor antagonist promethazine (10 μM, $n = 6$), atropine (10 μM, $n = 3$), *d*-tubocurarine (30 μM, $n = 3$) and the ATP P_2 receptor antagonist suramin (5 μM, $n = 4$) had no effect on bursting. These data suggest that bursting is mainly mediated by intrinsic, rather than chemical synaptic, mechanisms in SON neurones.

Hyperpolarization-activated cation current (I_H) and transient Ca^{2+} current (I_T)

Since many features of Ca^{2+}-free medium-induced bursting in SON neurones are similar to those of rhythmic activities described in other CNS neurones and generated by either or both I_H and I_T (Jahnsen & Llinás, 1984; McCormick & Pape,

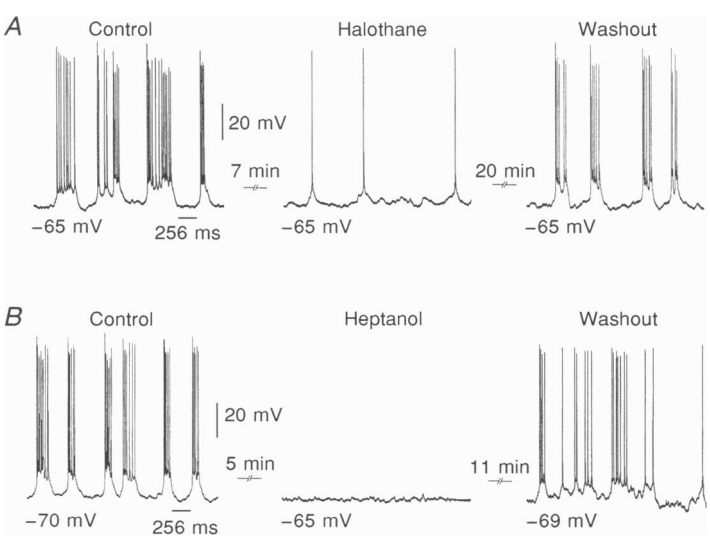

Figure 11. Uncoupling agents reversibly eliminated the bursting in 2 SON cells

All records were obtained during perifusion of slices with Ca^{2+}-free medium. *A* and *B*, voltage traces were obtained before (left), following perifusion of 2·5 mM halothane for 5·5 min (*A*, centre) or 1·7 mM heptanol for 4 min (*B*, centre), and 15 min (*A*, right) or 10 min (*B*, right) after the end of agent applications. Similar manipulation of membrane potential as shown in Figs 4 and 5 was performed in the present cases but again, for simplicity, only one expanded record during agent application is used to illustrate the abolishing effects on the oscillatory bursting. Note that increased negative current was injected through electrodes during agent application due to membrane depolarization, and that heptanol is distinguished from halothane by its effects of arresting action potentials (middle).

1990; Soltesz, Lightowler, Leresche, Jassik-Gerschenfeld, Pollard & Crunelli, 1991), specific blockers were used to see if these currents are responsible for the oscillatory bursting observed in the present experiments.

When the Ca^{2+}-free medium containing 2·5–4·0 mM Cs^+ was perifused for up to 20 min, an increase in membrane input resistance and hyperpolarization were observed, suggesting effective removal of I_H. However, these changes were not associated with abolition or reduction of bursting in any of the five neurones tested. Neither did bath application of Ni^{2+} (0·1–0·15 mM) alter burst activity ($n = 3/3$). These results suggest that both currents, although present in SON neurones, are not involved in generation of oscillatory bursting in SON cells.

DISCUSSION

In this study we found that membrane potential oscillations giving rise to an oscillatory burst activity emerged in SON magnocellular neurones in slices perifused with low-Ca^{2+} or Ca^{2+}-free medium. This oscillatory bursting was present in cells with DAPs and phasic firing, and prominent at membrane potentials between −50 and −70 mV. Addition of TTX or divalent cations into the Ca^{2+}-free medium, or replacement of external Na^+ with choline or Li^+ cancelled or reduced the oscillatory activity. Voltage-clamp experiments revealed a negative shift of activation threshold and enhancement of I_{NaP} during depletion of external Ca^{2+}. TEA or raised $[K^+]_o$ facilitated the bursting. Moreover, direct diffusion of BAPTA or heparin into cells or bath application of ryanodine suppressed the oscillatory bursting. Conventional uncoupling agents, such as heptanol, halothane and acidification, effectively abolished it. However, blockade of either chemical synaptic transmission with CNQX, APV, bicuculline, promethazine, atropine, *d*-tubocurarine and suramin, or other membrane currents (I_H and I_T) had no effect.

Involvement of Na^+ and K^+ in oscillatory bursting

Several lines of evidence obtained in the present study suggest that the oscillatory burst activity requires enhanced Na^+ influx. First, a specific Na^+ channel blocker (TTX) or reduced $[Na^+]_o$ eliminated the oscillations and bursting. Second, $[Ca^{2+}]_o$ influenced the oscillatory activity in a concentration-dependent manner; lower $[Ca^{2+}]_o$ always induced larger oscillation and bursts. Third, addition of other divalent cations that are known to mimic the effects of $[Ca^{2+}]_o$ on Na^+ channels (Moczydlowski & Schild, 1994) into the perifusion medium also blocked the bursting. Moreover, divalent cation concentrations needed to eliminate the oscillation were inversely related to their abilities to influence Na^+ channels; because of a lesser potency than Co^{2+}, Ni^{2+} or Ca^{2+}, a higher concentration of Mg^{2+} was required to block the bursting. In addition, abolition of bursting by TTX, but not by low concentrations of Ni^{2+}, also excludes the possibility that Ca^{2+}-activated non-specific cation channels and/or Ca^{2+} channels played critical roles in increased Na^+ influx observed in this study (for review see Partridge & Swandulla, 1988).

Based on activation threshold and functional roles in formation of action potentials, Na^+ currents can be divided into at least two types, i.e. I_{NaF} and I_{NaP} (Jahnsen & Llinás, 1984; Stafstrom *et al.* 1985). I_{NaF} has a higher threshold for activation, rapidly inactivates, and is responsible for fast depolarization and spike overshoot. I_{NaP} possesses a lower threshold, very slow activation and inactivation, and functions to bring the membrane potential to a level at which I_{NaF} can be activated. In cerebellar Purkinje cells, I_{NaP} produces an all-or-none, slow onset plateau potential lasting up to hundreds of milliseconds (Llinás & Sugimori, 1980). We found that perifusion of SON neurones with Ca^{2+}-free medium shifted I_{NaP} activation thresholds to more negative potentials and enhanced amplitudes of I_{NaP}. These changes in I_{NaP} were abolished by adding Co^{2+} or Mg^{2+} to Ca^{2+}-free medium. Because initial membrane depolarizations leading to bursts occurred at voltage levels close to I_{NaP} threshold and displayed a much longer rise time than that of superimposed fast spikes, it is suggested that I_{NaP} activation is essential for the onset of membrane oscillation.

Our observation of Na^+-dependent oscillatory bursting is consistent with an earlier report showing that treatment of SON magnocellular neurones with the Na^+ ionophore veratridine induces burst activity (Inenaga *et al.* 1993). However, these authors did not see any spontaneous oscillatory bursting in the Ca^{2+}-free medium like that observed in the present study. Although many factors, such as sharp electrode recording and slice preparation techniques, might contribute to this discrepancy, the high $[Mg^{2+}]_o$, which they used intentionally to block synaptic activities in their experiments, would also have blocked spontaneous oscillatory bursting.

Termination of the oscillatory bursts probably involves inactivation of the Na^+ currents, Na^+ accumulation resulting in raised $[Na^+]_i$ which reduces the driving force for Na^+ influx and/or enhanced activity of the Na^+–K^+ pump, moving Na^+ out of the cell. Faster membrane repolarization of oscillations observed in the present experiments than that of the veratridine-induced bursts (Inenaga *et al.* 1993) also hints at other mechanisms involved in terminating each membrane oscillation. K^+ involvement is supported by demonstration that either raising $[K^+]_o$ or application of low concentrations of TEA increased both oscillation amplitude and duration. This finding is not surprising because K^+ efflux, through voltage- and Ca^{2+}-dependent channels, has been ascertained to participate in repolarization of action potentials and plateau potentials in many neuronal preparations (Llinás & Sugimori, 1980). Replacement of Na^+ with either choline or Li^+, and low concentrations of TEA,

consistently induced membrane depolarizations. It is possible that Na^+ accumulation following bursts also triggers process(es) that repolarize SON cells. Whether a Na^+-dependent K^+ conductance, which has been identified in CNS neurones (Bader, Bernheim & Bertrand, 1985), is involved in shaping the bursting is unclear.

Dependence of bursting on internal Ca^{2+} mobilization and selective existence in different cell populations

Our observations that either intracellular diffusion of Ca^{2+} chelators or inhibition of Ca^{2+} release from internal stores eliminated or reduced the oscillatory activity with little influence on Na^+ current behaviour, suggest that rapid intracellular Ca^{2+} mobilization or oscillation is essential for burst generation and that internal stores are involved in regulation of $[Ca^{2+}]_i$ in SON neurones. Burst dependence on internal Ca^{2+} mobilization is consistent with studies showing that intracellular divalent cations increase the open probability of rat brain Na^+ channels and shift the gating curve to more negative potentials, effects which are opposite to that of external divalent cations (Moczydlowski & Schild, 1994). In addition, the observation that Ca^{2+}-dependent AHPs were still present following depletion of external Ca^{2+} (as shown in Fig. 9*B*) suggests that Ca^{2+} release from internal stores was enough to activate Ca^{2+}-dependent K^+ current, which could also participate in the termination of bursting. Whether Na^+ influx or depolarization can directly induce Ca^{2+} release in SON neurones, however, remains to be clarified (for review see Kuba, 1994).

On the basis of firing patterns and existence of DAPs, SON magnocellular neurones are divided into two different populations: those that fire prolonged bursts of action potentials interrupted by various durations of silence (i.e. phasic firing) and have DAPs, and those that continuously discharge spikes and do not display DAPs (Legendre & Poulain, 1992). Phasic firing is observed in vasopressinergic neurones and occasional oxytocinergic neurones, while most oxytocinergic neurones exhibit continuous activity (Armstrong *et al.* 1994). Our recent experiments have revealed that endogenous Ca^{2+}-binding proteins are responsible for the distinct electrophysiological features among SON neurones (Li *et al.* 1995). SON cells with stronger immunostaining for calbindin-D_{28k} most probably fire continuously and display no DAPs. Intracellular diffusion of antisera against this Ca^{2+}-binding protein converts continuous firing to phasic firing and reveals DAPs. However, SON cells with weaker calbindin-D_{28k} immunostaining usually generate Ca^{2+}-dependent phasic firing and DAPs, which can be eliminated by direct diffusion into the cell of calbindin-D_{28k} or BAPTA. In this study, removal of Ca^{2+} from perifusion medium was shown to induce oscillatory bursting in SON neurones possessing phasic firing and DAPs. Because of critical roles of internal Ca^{2+}, it is reasonable to conclude that less Ca^{2+}-buffering capacity contributes, at least in part, to selective existence of oscillatory bursting among certain SON neurones. This finding again supports the hypothesis that neuronal activity can be determined by endogenous mechanisms controlling $[Ca^{2+}]_i$.

Roles of gap junctions

Previous studies have provided evidence suggesting that gap junctions not only exist among SON magnocellular neurones but change in response to various physiological stimuli as well as to electrical stimulation of afferent fibres to the SON (Hatton, 1990). Electrical stimulation of afferent fibres from the tuberomammillary nucleus has recently been shown to enhance dye-coupling among phasically firing SON neurones, an effect which could be blocked by H_1-type histamine receptor antagonists or guanylyl cyclase inhibitors (Hatton & Yang, 1996). In the present study, several commonly used agents blocking gap junctional conductances cancelled bursting and induced membrane depolarization in SON neurones perifused with the Ca^{2+}-free medium. These results suggest involvement of metabolic and/or electrotonic coupling in Ca^{2+} depletion-induced oscillatory burst activity. Our findings are consistent with a recent observation in hippocampal CA1 neurones showing that Ca^{2+}-free medium increases the incidence of Lucifer Yellow dye-coupling, whereas uncoupling agents suppress the dye-coupling and eliminate a seizure-like oscillatory activity (Perez-Velazquez *et al.* 1994).

Although effective in blocking gap junctional transmission of electrical signals or dyes, common uncoupling agents have been reported to cause many other effects (Bennett *et al.* 1991). For example, they often suppress membrane Na^+ currents, including I_{NaP}, in the same concentration range as is needed to uncouple cells (Elliott *et al.* 1992). This is consistent with the present results showing that heptanol abolished Na^+-dependent fast spikes. That halothane and acidification cancelled the bursting with an increase in firing rate and that membrane depolarization was always induced by these uncoupling agents but never by TTX, however, suggest that suppression of Na^+ by common uncoupling agents is probably not a primary mechanism for blocking the oscillatory activity in SON neurones.

Gap junctions are important in synchronizing neuronal discharges to facilitate epileptiform activities (Llinás & Yarom, 1986; Christie *et al.* 1989). In recent years, spontaneous $[Ca^{2+}]_i$ oscillations and Ca^{2+} waves in the absence of extracellular Ca^{2+} have been observed in many kinds of cells (Schlegel *et al.* 1987; Boitano, Dirksen & Sanderson, 1992). Whether Ca^{2+} waves propagated through gap junctions, perhaps by transfer of IP_3, are involved in the oscillatory bursting observed in this study remains to be ascertained.

Comparison with oscillatory bursting in various preparations and functional significance

One important physiological feature of mammalian CNS neurones is the ability to generate rhythmic electrical oscillations (Llinás, 1988). The rhythmic activities or bursting have been seen in thalamocortical, thalamic, brainstem and thoracolumbar spinal cord neurones (Christie

et al. 1989; McCormick & Pape, 1990; Soltesz *et al.* 1991). However, almost all of these burst activities are known to involve increases in Ca^{2+} inward currents and/or I_H. Removal of extracellular Ca^{2+} elicits oscillatory bursting or epileptiform activity in the rat hippocampus, dentate gyrus and hypothalamus (Konnerth *et al.* 1986; Bouskila & Dudek, 1993; Perez-Velazquez *et al.* 1994). Although these electrophysiological events are found to be non-synaptically mediated and often require high $[K^+]_o$ to facilitate and spread them (Yaari, Konnerth & Heinemann, 1986), underlying ionic mechanisms are unknown. In the present experiments, we have demonstrated that one mechanism operating in the generation of oscillatory bursting by depletion of external Ca^{2+} involves enhancement of I_{NaP}. This is probably due to low $[Ca^{2+}]_o$ increasing the availability of negative surface charges on the outside of cell membranes and reducing inhibition of Na^+ currents. We have also provided preliminary evidence supporting the roles of intracellular Ca^{2+} mobilization in generation of the oscillatory bursting. The present work may be of significance in understanding ionic mechanisms for epileptogenesis in the brain, where low external Ca^{2+} is often revealed, and in *in vitro* epileptic models. Our findings, in particular the dependence of Na^+ influx through persistent Na^+ channels on $[Ca^{2+}]_i$, and selective existence of bursting, provide new insights into investigation of the generation of DAPs and phasic firing patterns in SON magnocellular neurones.

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