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BURSTING PROPERTIES OF CAUDAL NEUROSECRETORY CELLS IN THE FLOUNDER *PLATICHTHYS FLESUS, IN VITRO*

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Summary

Bursting activity in type 1 Dahlgren cells was studied using intra- and extracellular recording from an *in vitro* preparation of the caudal neurosecretory system of the euryhaline flounder. 45 % of cells showed spontaneous bursts of approximately 120 s duration and 380 s cycle period. Similar bursts were triggered by short duration (<5 s) depolarising or hyperpolarising pulses. Cells displayed a characteristic depolarising after potential, following either an action potential with associated afterhyperpolarisation, or a hyperpolarising current pulse. This depolarising after potential was related to a 'sag' potential, which developed during the hyperpolarising pulse. Both the depolarising after potential and the sag potential occurred only in cells at more depolarised (<60 mV) holding potentials. In addition, the amplitude of the depolarising after potential was dependent on the amplitude and the duration of the hyperpolarising pulse. The depolarising after potential following action potentials may provide a mechanism for facilitating repetitive firing during a burst. Extracellular recording revealed similar bursting in individual units which was not, however, synchronised between units. Spontaneous bursting activity recorded both intra- and extracellularly was inhibited by application of a known neuromodulator of the system, 5-hydroxytryptamine. This study provides a basis for investigating the relationship between physiological status, Dahlgren cell activity and neuropeptide secretion.

Key words: neurosecretion, bursting, electrophysiology, flounder, *Platichthys flesus*, osmoregulation.

Introduction

The caudal neurosecretory system (CNSS) of teleost fish (including the European flounder *Platichthys flesus*) is a discrete and accessible structure, with many features comparable to those of other, cephalic vertebrate neuroendocrine systems. The CNSS consists of large magnocellular neurons (Dahlgren cells) located in the terminal vertebral segments of the spinal cord, which project primary axons to a discrete neurohaemal organ, the urophysis (Arnold-Reed et al., 1991; Hubbard et al., 1996b). Dahlgren cells synthesise and secrete two unrelated peptide hormones, urotensins I (UI) and II (UII) (Pearson et al., 1980; Lederis et al., 1982), the biological actions of which indicate a role in osmoregulation and ion homeostasis (Bern et al., 1985; Lederis et al., 1985). In support of this, we have recently shown, using an homologous radioimmunoassay, that plasma concentrations of UII are significantly elevated in seawater- compared to freshwater-adapted flounder (Winter et al., 1999). Furthermore, the urophysial content of neurosecretory material becomes depleted in response to hyperosmotic challenge (Arnold-Reed et al., 1991).

The CNSS receives descending input, largely from the hindbrain (Cohen and Kriebel, 1989). Immunohistochemical and biochemical studies indicate adrenergic, serotonergic, cholinergic and peptidergic (gonadotropin-releasing hormone and neuropeptide Y) inputs to the CNSS (Audet and Chevalier, 1981; McKeon et al., 1988; Miller and Kriebel, 1986; Yulis et al., 1990; Oka et al., 1997). The origin and physiological role of these inputs is unknown. We have begun to characterise the actions of specific neurotransmitters/modulators (monoamines, acetylcholine) on Dahlgren cells in an *in vitro* CNSS preparation from the euryhaline flounder (Hubbard et al., 1996a; Hubbard et al., 1997; Brierley et al., 2000). Descending input to the Dahlgren cells is likely to influence the electrical output of the CNSS by modulating both intrinsic cellular and local network properties and, hence, to affect patterns of peptide secretion. However, the spontaneous firing patterns of type 1 Dahlgren cells have not yet been described in detail.

Two subtypes of Dahlgren cell (type 1 and type 2) have been identified in seawater-adapted flounder using electrophysiological criteria (Hubbard et al., 1996b). It is unlikely that these represent separate populations secreting either UI or UII, since immunocytochemical studies indicate that the two peptides are colocalised in >90 % of all Dahlgren cells (Larson et al., 1987; A. Ashworth, unpublished). Type 2 cells are electrically silent in the *in vitro* system and can only

be induced to fire single action potentials, during depolarising current injection, owing to spike frequency accommodation (Hubbard et al., 1996b). In contrast, the more numerous type 1 cells are usually spontaneously active, often generating characteristic bursting activity. The ability to generate prolonged high frequency bursts of spikes is a common feature of secretory cells in both vertebrates and invertebrates (e.g. mammalian hypothalamic neurons, Lincoln and Wakerley, 1974; pancreatic β-cells, Bertram and Sherman, 2000; *Aplysia brasiliana* R15 neuron, Dudek et al., 1979). Each burst of electrical activity facilitates the release of a bolus of neuropeptide in sufficient quantity transiently to increase circulating levels. Bursting properties depend on intrinsic neuronal properties or on local network interactions, or may reflect a combination of the two. Furthermore, the modulation of the intrinsic burst properties of the Dahlgren cells is likely to be reflected in changes in neuropeptide secretion *via* the urophysis.

The European flounder is of marine origin and is one of the few fish species that can fully adapt its osmoregulatory physiology to both sea and fresh water. In this study, we have exploited the highly accessible *in vitro* preparation of the CNSS of seawater-adapted flounder, in order to characterise bursting parameters of type 1 Dahlgren cells in the absence of external inputs. The aims were, firstly, to define intrinsic cellular parameters of Dahlgren cell bursting activity that are likely to be modulated by descending input to the CNSS during the process of physiological adaptation, thus leading to altered peptide secretion *in vivo*. This was achieved using intracellular recordings from individual type 1 Dahlgren cells. Secondly, extracellular recordings were carried out to determine the relative firing patterns of groups of Dahlgren (presumably type 1) cells and, in particular, whether their bursting activity is synchronised or coordinated. Thirdly, experiments were carried out to determine whether a known neuromodulator substance could influence ongoing bursting activity in Dahlgren cells. 5-Hydroxytryptamine (5-HT) was chosen for these experiments since this neuromodulator has been shown to be present in the CNSS (Cohen et al., 1990) and to hyperpolarise Dahlgren cells directly (Hubbard et al., 1997).

Materials and methods

Adult flounder *Platichthys flesus* L (300–800 g), caught in the Lune and Dee estuaries, were kept under a 12:12 h light:dark cycle in sea water (8–10 °C) for at least 14 days to allow full osmotic adaptation before experimentation. Fish were killed in accordance with UK licensed procedures (Home Office Schedule 1); the terminal portion of the spinal cord (7–10 vertebral segments) with urophysis attached was dissected out, and the connective tissue sheath around the cord carefully removed. The spinal cord was placed in a cooled interface recording chamber (12–15 °C), continuously superfused with cold (8–12 °C), aerated seawater-flounder Ringer (composition in mmol l^{-1}: K_2HPO_4 1.0, KCl 0.5, NaCl 155, $NaHCO_3$ 10, $CaCl_2$ 2.12, $MgSO_4$ 1.0, D-glucose 5.56, pH 7.7) at 0.6 ml min^{-1}. Tissue was allowed to equilibrate for 1 h before recordings were made.

Intracellular recordings of Dahlgren cell activity

Intracellular recordings were made from individual Dahlgren cells using glass microelectrodes filled with 3 mol l^{-1} potassium acetate with resistances of 50–100 MΩ. The electrode was connected to an Axoclamp 2A amplifier (Axon Instruments, CA, USA), data captured *via* a CED 1401 converter (Cambridge Electronic Design, UK) and stored and analysed using CED SIGNAL version 1.72 and Spike2 version 2.01 software. Once a cell was penetrated, the bridge-balance facility in the amplifier was adjusted whilst passing 500 ms, −0.3 nA pulses at 0.3 Hz. Only cells that maintained a steady resting membrane potential of at least −50 mV, and that could generate overshooting action potentials, were considered viable. Type 1 cells were impaled and held for up to 120 min, and their spontaneous activity was recorded continuously. Responses to hyperpolarising current pulses (50–1000 ms, −0.1 to −1.5 nA) were recorded. In addition, depolarised and hyperpolarised membrane potentials could be imposed by continuous current injection in order to examine the response to current pulses at different holding potentials.

Extracellular recordings from the CNSS

Extracellular recordings were made under bath conditions similar to those described above, using either two fine silver wire hook electrodes (0.5 mm diameter wire, flattened to approx. 1 mm to increase the area of contact, 1 mm apart) placed under the spinal cord, or a suction electrode with pipette diameter approx. 0.5 mm, at pre-terminal segments 2 or 3. Stable recordings could be maintained for more than 6 h. Signals were recorded differentially and amplified using a Neurolog AC NL104 amplifier (Digitimer, UK) and filtered (AC NL125; 5 Hz and 1.2 kHz cut-off frequencies plus a 50 Hz notch filter). A CED converter was used to digitise the signals and CED Spike2 software for storage and analysis.

Bath and focal application of 5-HT

Both focal (during intracellular recordings) and bath (for extracellular recordings) drug applications were achieved using simple gravity-fed systems with a 10 ml and 50 ml reservoir, respectively. Focal applications (2–10 s) of 5-HT (100 μmol l^{-1}) *via* large diameter glass electrodes (placed 100–500 μm upstream of the recording site) restricted 5-HT applications to the spinal cord segment from which recordings were being made. The perfusion of the spinal cord in normal Ringer was maintained throughout. In contrast, during extracellular recording, the whole CNSS was superfused with 5-HT (100 μmol l^{-1}). The preparation was superfused with normal Ringer for 2 h prior to any 5-HT application. The CNSS was then superfused with 5-HT and allowed to equilibrate in this medium for 30 min, after which it was returned to normal Ringer for up to a further 3.5 h.

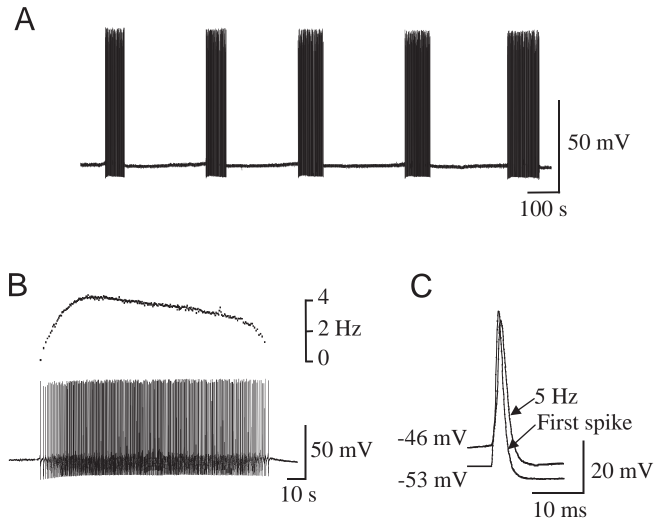

Fig. 1. Bursting patterns in type 1 Dahlgren cells. (A) Spontaneous bursting pattern. (B) A single burst (bottom trace) and instantaneous spike frequency (top trace) revealing acceleration and deceleration phases with a peak firing frequency of approx. 4 Hz. (C) Action potential duration (measured at half-maximal amplitude) increases with instantaneous spike frequency. At maximal firing rate, spike duration increases (approximately 30 %) compared to the first spike of the burst.

Table 1. *Burst parameters from spontaneously bursting Dahlgren cells*

Burst parameter	Mean ± S.E.M.
Burst duration (s)	124±13.8
Cycle period (s)	382.2±49.4
Acceleration phase (s)	28.7±3.5
Deceleration phase (s)	95.2±11.7
Spikes per burst	324±40
Maximum spike frequency (Hz)	4.5±0.4
Mean spike frequency (Hz)	2.6±0.2

Values are means (N=14) of means of more than three bursts for each cell.

Results

Characterisation of spontaneous electrical activity in Dahlgren cells

During intracellular recording from single cells, type 1 Dahlgren cells were identified according to electrophysiological criteria described by Hubbard et al. (Hubbard et al., 1996b). Specifically these were their firing response to current injection and their action potential waveform, and in particular the large amplitude and long-duration spike afterhyperpolarisation (AHP). 31 cells, from 31 preparations, were identified as type 1 Dahlgren cells, of which 14 (45 %) generated spontaneous bursting activity. The remaining 17 cells were either silent (16 %) or generated tonic firing activity (39 %). Type 1 cells had resting membrane potentials of –61.4±1.5 mV (mean ± S.E.M.). Tonically active cells had a mean instantaneous firing frequency of 2.6±0.5 Hz (N=12). Quiescent type 1 cells could be depolarised supra-threshold for action potential generation by relatively small amounts of current injection (<0.3 nA). This, together with the waveform of the resulting action potential, distinguished them from type 2 neurons. The characteristics of spontaneous bursting activity in the remaining type 1 Dahlgren cells were examined further.

Bursting properties of Dahlgren cells

Bursts of action potentials lasted approx. 120 s, and were separated by periods of inactivity lasting between 70 s and 600 s (Fig. 1A), yielding a mean cycle period of 382±49.4 s. Burst duration and total number of spikes per burst were remarkably consistent both within and between cells *in vitro* (Table 1), suggesting at least some intrinsic or local component to burst generation. Fig. 1B shows a typical burst together with a plot of instantaneous spike frequency. There is an initial acceleration phase (to a maximum frequency of 4.5±0.4 Hz after 28.7±3.5 s), followed by slower deceleration and burst termination. The time taken and the number of spikes generated to reach maximum spike frequency were again consistent within and between cells, as were the parameters relating to the deceleration phase (Table 1).

Action potential shape and instantaneous spike frequency

The duration of action potentials (measured at half-peak amplitude) increased during the acceleration phase of the burst. Spike duration was 2.6±0.4 ms for the first spike of the burst and increased significantly, by 30 %, to 3.4±0.5 ms (N=6 cells, P<0.01, one-way ANOVA plus Dunnett's multiple comparison test) by the time spike frequency reached 5 Hz (Fig. 1C). Similarly spike AHP decreased in amplitude with increased firing rate; maximum membrane potential achieved at the trough of the AHP decreased by 5.0±0.6 mV. This change in shape was related to the instantaneous spike frequency and spike shape returned to its initial value by the end of the burst.

Spontaneous and evoked bursts

During generation of spontaneous bursting, bursts appeared to be triggered by a compound excitatory postsynaptic potential (EPSP), which led to depolarisation of the membrane by 7.0±0.7 mV (N=14 cells) until action potential threshold was reached (Fig. 2). The compound EPSP consisted of a slow wave of depolarisation, upon which were superimposed fast, possibly unitary, EPSPs of 2–4 mV amplitude and 30 ms duration. The origin of these inputs in this *in vitro* preparation is unknown.

Prolonged bursts of activity, resembling spontaneous bursts, were reliably triggered by injection of a brief, 2–5 s depolarising pulse (0.5–1.0 nA) through the microelectrode (Fig. 3A), suggesting that a local or intrinsic burst-generating mechanism was activated in the absence of prolonged synaptic input. In a small number of cells, it was possible to trigger a relatively short burst on the rebound from an injected hyperpolarising pulse. An example is shown in Fig. 3B, in

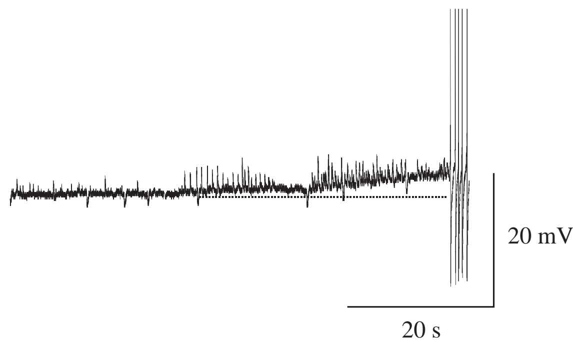

Fig. 2. Spontaneous depolarising input to type 1 cells. Prior to the onset of a burst, a depolarising waveform is seen, consisting of at least two excitatory components. Underlying the fast unitary events is a slower depolarisation, which reaches spike threshold after approximately 35 s.

which a 500 ms (−0.9 nA) hyperpolarising current triggered a burst of spikes lasting 55 s. Closer inspection of spike initiation triggered by a hyperpolarising current pulse revealed the presence of a depolarising after potential (DAP, Fig. 3C), similar to that reported for other bursting neurons (Legendre and Poulain, 1992). In some cases this was able to initiate more than one action potential on the rebound from hyperpolarisation. The voltage- and time-dependence of DAP generation was examined further.

Voltage- and time-dependent generation of depolarising after potentials

Depolarising after potentials were only observed in cells with the resting membrane potential depolarised above approx. −60 mV, and could trigger action potentials when the resting potential was approx. −50 mV. In cells in which the membrane potential was held at −50 mV by continuous current injection, further injection of a 500 ms hyperpolarising pulse led to a hyperpolarising response with a marked depolarising 'sag', suggesting activation of a slow inward current. Fig. 4A illustrates the time course of this sag potential, which reached a maximum after 200 ms and then remained for the duration of the current pulse. The offset of the pulse was followed by a membrane potential rebound, or DAP, of 3–8 mV amplitude and 300–500 ms duration (Fig. 4A). The amplitudes of the sag potential and DAP in response to a hyperpolarising pulse (−1.2 nA) were measured in cells held at three different holding potentials. Both were greater at the more depolarised potential and were negligible when the holding potential was hyperpolarised beyond −65 mV (Fig. 4A). Furthermore, their amplitude was dependent on the size of the hyperpolarising current pulse; an increase in current injection from a given holding potential led to an increase in the amplitude of DAP (Fig. 4B). The amplitude of the DAP was also dependent on the duration of the hyperpolarising current pulse for pulses ranging between 50 and 400 ms, with increased pulse duration yielding an increased DAP (Fig. 4C).

Inspection of action potentials at the onset of a burst revealed the typical large-amplitude AHP of type 1 Dahlgren

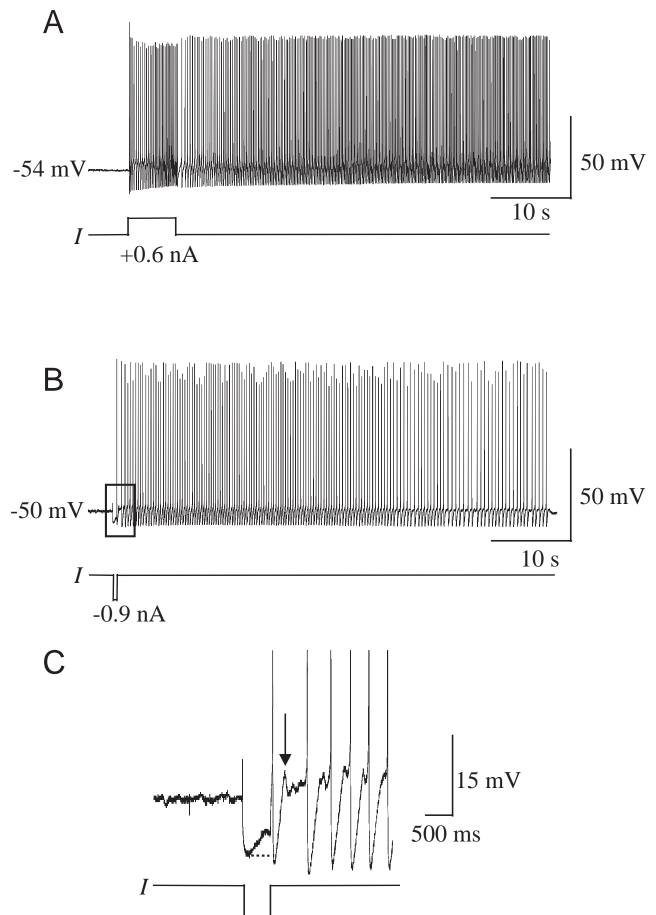

Fig. 3. Burst triggering by current injection. (A) Injection of a 5 s depolarising pulse *via* the recording electrode (during the first half of an interburst interval) triggered a burst, which lasted 128 s (end of burst not shown). (B) Following termination of a 500 ms hyperpolarising pulse, a burst of action potentials, lasting 55 s, occurred. The same hyperpolarising pulse and first six spikes (highlighted in the box) are shown on an expanded scale in (C). During the pulse, the membrane potential waveform does not follow that of the square wave hyperpolarisation (deviation from dashed line) and upon termination of the pulse, an action potential is immediately triggered. Succeeding this action potential, but prior to the onset of the next, is a depolarising after potential (DAP; arrow).

cells of between 10 and 20 mV, which is comparable to the initial voltage response to a hyperpolarising pulse (−1.2 nA; Fig. 4A). This was followed by a depolarising overshoot (arrow, Fig. 4D), which probably represents the DAP rebound following hyperpolarisation, and which may provide an intrinsic spike triggering mechanism facilitating repetitive firing during a burst.

Extracellular recording from Dahlgren cells

Extracellular recording from the spinal cord (*N*=17 preparations) enabled the simultaneous monitoring of activity of a number of Dahlgren cells, which could often be separated by differences in apparent spike amplitude (Fig. 5A). Where activity was discernible from a single

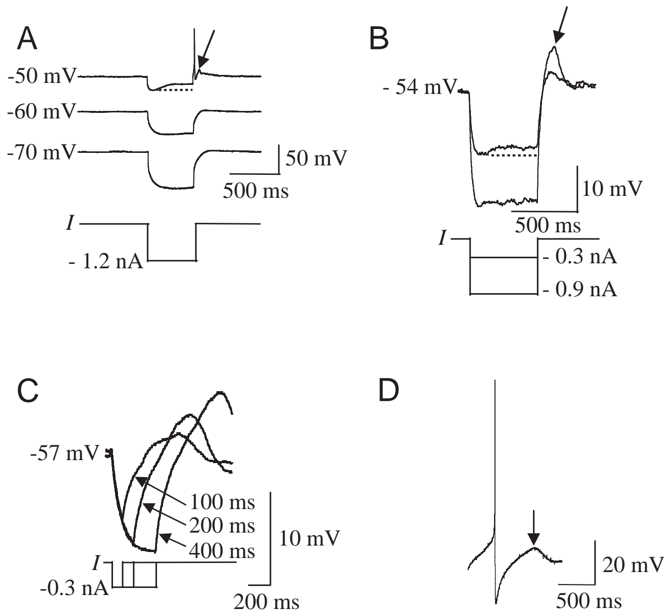

Fig. 4. Voltage- and time-dependence of depolarising after potentials. (A) The membrane potential (E_M) of a type 1 cell was held at three different potentials by constant current injection. Constant amplitude and duration hyperpolarizing pulses elicit different membrane potential waveforms, depending on E_M. A sag potential (dashed line) occurs during the pulse and a spike is triggered on pulse termination only at the more depolarised E_M. A DAP (arrowed) follows the action potential. Membrane sag and DAP are thus dependent on E_M. (B) Hyperpolarising pulses of constant duration but differing amplitude were injected into a depolarised type 1 cell. DAP amplitude increases with increased hyperpolarising pulses. (C) Constant amplitude hyperpolarizing current pulses of increasing duration were applied to a type 1 cell. DAP amplitude increase with increased pulse duration. (D) The first spike of a spontaneous burst is succeeded by a DAP (arrow).

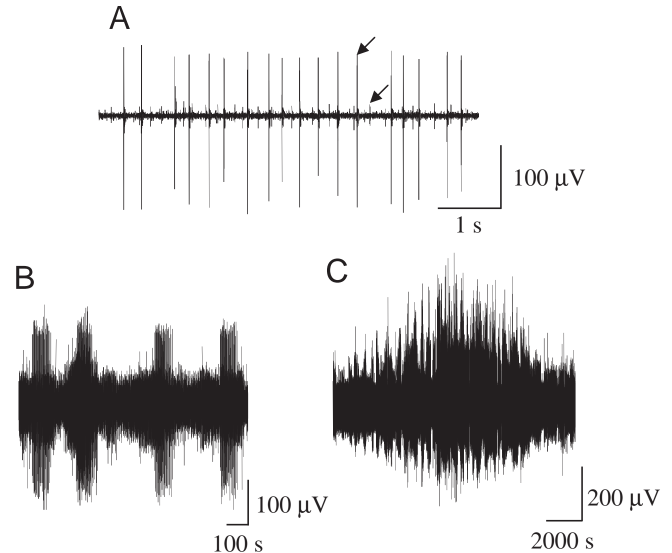

Fig. 5. Extracellular recordings of CNSS activity. (A) Individual units (arrows) can be distinguished by spike amplitude. (B) Bursts of activity (approximately 120 s duration) are seen in an individual unit. (C) Activity from several units contributes to a long-duration superburst.

Dahlgren cell it was possible to distinguish bursting activity with burst durations (approx. 120 s) comparable to those displayed by single cells recorded intracellularly (Fig. 5B). The most notable observation was that Dahlgren cells did not burst synchronously. Instead, the combined activity of a small population of Dahlgren cells often contributed to much longer ‘superbursts’, whose duration varied from 500 to 900 s (Fig. 5C).

Inhibition of spontaneous activity by 5-hydroxytryptamine

Serotonergic innervation of the CNSS (McKeon et al., 1988) and hyperpolarisation of type 1 cells by 5-HT (Hubbard et al., 1997) have both been reported. In this study we examined the effect of 5-HT on bursting in Dahlgren cells. Focal application of 10^{-4} mol l^{-1} 5-HT adjacent to the recording site reliably and abruptly terminated spontaneous bursts recorded intracellularly, followed by prolonged hyperpolarisation by up to 20 mV (*N*=6) (Fig. 6A). Similarly, during extracellular recording, superfusion of 5-HT (10^{-4} mol l^{-1}) abolished activity in all cells recorded, with activity resuming after >20 min (*N*=5) (Fig. 6B).

Discussion

This study is the first to report spontaneous bursting activity for Dahlgren cells in the teleost CNSS. Previous electrophysiological studies in this and other species concentrated on action potential parameters, and responses to current injection and extracellular stimulation (Bennett and Fox, 1962; Hubbard et al., 1996a). Bursting activity is a common property of many neurosecretory neurons (e.g. mammalian hypothalamic magnocellular neurons; reviewed by Poulain and Wakerley, 1982; Legendre and Poulain, 1992). Indeed, it has been shown that bursts, as opposed to tonic firing, lead to more effective peptide secretion (e.g. Cazalis et al., 1985) owing to ‘frequency facilitation’, in which, as firing rate increases, the amount of peptide released from axon terminals also increases. This is thought to result both from spike broadening (Jackson et al., 1991), as seen here in Dahlgren cells, and from a progressive rise in extracellular [K$^+$] around the terminals leading to persistent depolarisation during the burst (Leng and Brown, 1997). Furthermore, it was proposed, for magnocellular oxytocin cells, that synthesis of peptide is also linked to electrical activity, leading to the accumulation of stored hormone when secretion is suppressed (Leng et al., 1993). Individual type 1 Dahlgren cells are capable of generating discrete, tightly regulated bursts, each of which presumably leads to the release of a bolus of secretory product (urotensins) from the urophysis. These bursts are generated by at least a proportion of cells *in vitro* showing that the generation of bursts is controlled by mechanisms local to the CNSS, though modulation by descending inputs is likely. Our data point to intrinsic (i.e. cellular) properties underlying bursts of spikes. First, bursts were relatively homogeneous both within and between cells, especially with regard to their

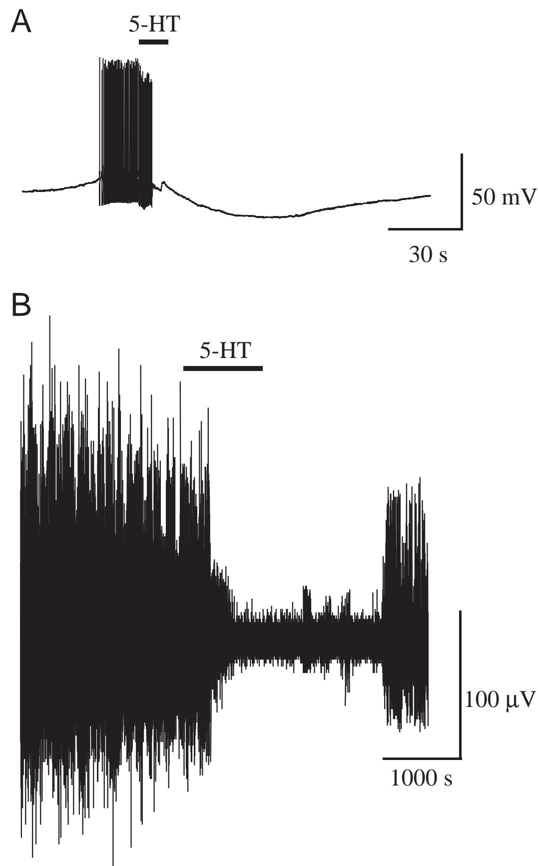

Fig. 6. Burst termination by 5-HT. (A) A spontaneous burst recorded intracellularly in a type 1 Dahlgren cell is prematurely terminated by 10 s focal application of 5-HT (100 µmol l^{-1}; bar), which also hyperpolarised the cell by 25 mV. (B) A 15 min bath application of 5-HT (100 µmol l^{-1}; bar) abolishes ongoing activity. Spontaneous activity returns following a 25 min washout.

duration and maximum firing rate. Second, it was possible to trigger similar bursts following brief depolarising or hyperpolarising stimuli, indicating that Dahlgren cells possess properties that facilitate bursting. At least one of these properties is the DAP that follows hyperpolarising current injection and is also apparent following spike AHP. Depolarising after potentials and associated inward sag potentials have been described for other neurosecretory neurons. For example, mammalian supraoptic (vasopressin) neurons show inward rectification (probably Ca^{2+}-dependent) in response to a hyperpolarising current pulse (Erickson et al., 1990).

The DAP provides a potential means for maintaining repetitive firing since each action potential and associated AHP is followed by a rebound depolarisation, which may reach threshold. In the Dahlgren cells, the sag potential took around 200 ms to develop; this is similar to the duration of the AHP and could account in part for the maximum spike frequency of 5 Hz seen during a burst. Another limit to firing rate within a burst was action potential shape. At maximum firing frequency, spike duration increased and AHP amplitude reduced. The latter would lead to decreased activation of the inward current underlying DAP and thus delay the subsequent action potential, leading to a slowing of firing rate in the latter part of the burst. The occurrence of DAP was dependent on membrane potential; it appeared only in relatively depolarised cells. This suggests that bursting is, at least partially, dependent on background excitation, either from descending pathways or local interneuronal networks. Even in our isolated, *in vitro* preparation, bursts appeared to be triggered by excitatory synaptic inputs, which must have been generated locally. A population of local serotonergic interneurons was identified within the CNSS of the molly *Poecilia latipinna* (Cohen et al., 1990), which may form part of a rhythm generating network, providing phasic input to Dahlgren cells. However, since 5-HT is inhibitory to these cells, excitatory neurons, possibly cholinergic, must also be involved. Acetylcholine is present in large amounts in the CNSS of teleosts (Conlon and Balment, 1996) and has been shown in preliminary experiments to excite flounder Dahlgren cells (Brierley et al., 2000). The mechanism underlying burst termination is unknown since spike duration and AHP returned to their original level as firing rate declined.

Simultaneous extracellular recording from a group of Dahlgren cells showed no evidence for synchronised bursting. In contrast, close coupling of magnocellular oxytocin neurons leads to synchronised population bursts which have been linked to pulsatile release of high concentrations of peptide for phasic milk ejection (Poulain and Wakerley, 1982). These bursts have high frequency and short duration compared to those recorded in Dahlgren cells, typically comprising 70–80 spikes within 2–4 s. The asynchronous bursting activity of the Dahlgren cells resembles more that of vasopressin hypothalamic neurons, with longer and less intense bursts. During spontaneous phasic activity, the latter show burst durations of up to 100 s and intraburst firing rates up to 15 spikes s^{-1}, with no synchronisation of bursting activity between cells (Poulain and Wakerley, 1982). Thus, for vasopressin neurons, and probably Dahlgren cells, the bursts provide an efficient means of secreting hormone, whilst asynchrony of bursts ensures continuous rather than pulsatile output. The lack of apparent coupling between the activity of Dahlgren cells does not, however, rule out the possibility of local interactions at the level of the neurosecretory axon terminals. Cioni and De Vito (Cioni and De Vito, 2000) reported colocalisation of UI and UII with nitric oxide synthase in axon terminals in the CNSS of the teleost *Oreochromis niloticus*. This raises the possibility that nitric oxide produced in response to neuronal activity could act locally to modulate further release of urotensins. The occurrence of the ‘superbursts’ recorded extracellularly in Dahlgren cells may have functional significance or may represent an artefact owing to the relatively small number of cells being sampled.

Pharmacological concentrations of 5-HT terminated individual bursts and inhibited bursting in the Dahlgren cell population, showing that experimental manipulation of the activity of the system using known neuromodulators is achievable. As suggested by Poulain and Wakerley (Poulain

and Wakerley, 1982), it is likely that neurosecretory cell activity would be modulated in two ways by input pathways. Subtle modifications of peptide secretion are probably achieved by alterations in burst frequency and duration, via modification of intrinsic parameters that facilitate bursts, such as membrane potential, AHP and DAP. However, the amount of peptide secreted might also depend on recruitment of silent or tonically active cells. Like Dahlgren cells, vasopressin neurons show three different activity patterns (slow irregular, fast continuous and phasic), which probably represent different activity states of the same neuron type (Poulain and Wakerley, 1982).

Bursting activity in Dahlgren cells is relatively homogeneous in the *in vitro* system, and will enable future characterisation of factors that influence CNSS activity and urotensin secretion. Considerable variation in the bursting pattern must occur *in vivo*, in response to changes, for example, in osmotic status. Both intra- and extracellular recording from the CNSS of flounder *in vivo* are feasible, and will allow us to investigate the functional role of this discrete neurosecretory system at the cellular level within its physiological context.

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