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REVIEW

DORSAL UNPAIRED MEDIAN NEURONES IN THE INSECT CENTRAL NERVOUS SYSTEM: TOWARDS A BETTER UNDERSTANDING OF THE IONIC MECHANISMS UNDERLYING SPONTANEOUS ELECTRICAL ACTIVITY

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Summary

The efferent dorsal unpaired median (DUM) neurones, which include octopaminergic neurones, are among the most intensively studied neurones in the insect central nervous system. They differ from other insect neurones in generating endogenous spontaneous overshooting action potentials. The second half of the 1980s is certain to be considered a turning point in the study of the ion channels underlying the electrical activity of DUM neurones. Recent advances made using the patch-clamp technique have stimulated an increasing interest in the understanding of the biophysical properties of both voltage-dependent and voltage-independent ion channels. Patch-clamp studies of DUM neurones in cell culture demonstrate that these neurones express a wide variety of ion channels. At least five different types of K^+ channel have been identified: inward rectifier, delayed rectifier and A-like channels as well as Ca^{2+}- and Na^+-activated K^+ channels. Moreover, besides voltage-dependent Na^+ and Ca^{2+}-sensitive Cl^- channels, DUM neurones also express four types of Ca^{2+} channel distinguished on the basis of their kinetics, voltage range of activation and pharmacological profile. Finally, two distinct resting Ca^{2+} and Na^+ channels have been shown to be involved in maintaining the membrane potential and in regulating the firing pattern. In this review, we have also attempted critically to evaluate these existing ion channels with regard to their specific functions in the generation of the different phases of the spontaneous electrical activity of the DUM neurone.

Key words: insect, dorsal unpaired median neurones, pacemaker activity, electrophysiology, ionic current.

Introduction

The electrical activity of the dorsal unpaired median neurone: past directions

Historically, many electrophysiological studies performed *in vivo* indicated that the somata of some insect neurones were electrically inexcitable (Hoyle and Burrows, 1973) or displayed only attenuated action potentials (Crossman et al., 1971) that corresponded, sometimes, to electrical activity propagated electrotonically from more or less distant regions of active membrane. However, a distinct population of neurones, with somata located along the dorsal midline of many ganglia of the insect ventral nerve cord, constituted a notable exception to this rule. These neurones, which made symmetrical left and right branches, were originally described by Plotnikova (1969) in locusts and were later named dorsal unpaired median (DUM) neurones (Hoyle et al., 1974). Like many other insect neurone somata located in the ganglion, DUM neurone cell bodies (45–60 μm in diameter) were readily accessible and easily penetrated with intracellular microelectrodes. The first few reports indicating that the action potentials of DUM neurones were generated intrasomatically came from studies performed on cell bodies located on the dorsal surface of the terminal abdominal ganglion (TAG) of the cockroach *Periplaneta americana* (Callec and Boistel, 1966; Kerkut et al., 1968; Jego et al., 1970; Crossman et al., 1971). These electrophysiological studies proved that the cell bodies were capable of generating spontaneous overshooting action potentials and that Na^+ was responsible for the depolarizing phase of the action potentials (Jego et al., 1970).

A substantial number of electrophysiological investigations over the last 20 years have demonstrated an unexpected complexity regarding the electrophysiological properties of DUM neurones. The progeny of the median neuroblast (see Goodman, 1982; Boyan and Ball, 1993; Condron and Zinn, 1994) displayed a broad spectrum of electrical properties related to their neural geometry. It was reported, particularly in cockroach and locust, that adult DUM neurones exhibited

overshooting spiking somata from both neurites and axons, that some of them had non-spiking somata with both spiking axons and neurites, and that another type of DUM neurone had only spiking axons (Crossman et al., 1972; Heitler and Goodman, 1978; Hoyle and Dagan, 1978; Goodman et al., 1980; Thompson and Siegler, 1991). Overshooting spontaneous somatic action potentials were found only in a special group of DUM neurones identified as modulatory efferent DUM neurones (i.e. neurones having two symmetrical neurites that run to specific peripheral nerves on each side of the ganglion) (for morphology, see Watson, 1984; Burrows, 1996). This review focuses on the electrical activity of efferent DUM neurones. Two other groups of neurones, including local DUM interneurones and intersegmental DUM interneurones, have only passively conducted attenuated action potentials or are nonspiking (Goodman et al., 1980; Thompson and Siegler, 1991). In the latter case, the neurone normally did not produce action potentials. When some of the outward current was blocked by tetraethylammonium chloride (TEA-Cl), the cell produced a Ca^{2+}-dependent spike unaffected by the removal of Na^+ but completely blocked by Co^{2+} (Goodman et al., 1980).

Because efferent DUM neurone somata all cluster in a distinct area and appear to be encased in a common glial sheath, it was supposed that their cell bodies might be electrically or synaptically coupled. Electrophysiological studies performed during locust embryogenesis provided a better understanding of the embryonic development of electrical excitability in DUM neurones. In early locust embryos (e.g. on day 10), some neuronal precursor cells (e.g. median neuroblast) and their first-born identified progeny had linear current–voltage relationships and appeared to be both electrically inexcitable (apparent input resistance greater than 200 MΩ) and highly electrically coupled (Goodman and Spitzer, 1981b). The site of electrical coupling seemed to be directly between cell bodies because the neurones at this early stage lack axons (Goodman, 1982). During embryogenesis (at approximately day 12), these DUM neurones acquired the ability to generate overshooting action potentials characterized by a pronounced afterhyperpolarization. Between days 10 and 13, the coupling disappeared and the cell bodies became electrically uncoupled from each other (Goodman and Spitzer, 1979, 1981b). During this period, the two events (coupling and excitability) were independent and occurred at the same developmental stage. In other words, the electrical coupling was not masking the temporal sequence of appearance of voltage-dependent ionic currents during the differentiation of the cells. By day 18, most DUM neurones had attained their mature electrophysiological properties. The somata generated overshooting action potentials with an amplitude of 80–110 mV (Goodman et al., 1980; Goodman and Spitzer, 1981a,b). The recorded resting potential varied from −45 to −60 mV, and the cell bodies showed strong TEA-Cl-sensitive delayed rectification when depolarized. The input membrane resistance also decreased from more than 200 MΩ to approximately 10 MΩ. This reflected (i) an increase in membrane surface area, (ii) a decrease in specific membrane resistance and (iii) a change in the relative density of the ionic channels.

The overshooting soma action potentials appearing on day 12 were carried by both Na^+ and Ca^{2+} because the removal of Na^+ or the addition of Co^{2+} failed to block the action potentials, whereas simultaneous application of both treatments did. Additional experiments performed on adult identified locust DUM neurones (e.g. DUMETi) reported that replacing Na^+ with choline or the addition of tetrodotoxin (TTX) progressively eliminated soma spikes. Addition of Co^{2+} or La^{3+} also abolished soma spikes. These results indicated that the inward currents carried by Na^+ and Ca^{2+} involved different ionic channels. In contrast, Jego et al. (1970) reported that the inward current of the soma action potential recorded from unidentified spiking somata located on the dorsal surface of the cockroach TAG was carried predominantly by Na^+. Even though these authors did not directly address the issue of the requirement for Ca^{2+} in their study, this result was confirmed both *in situ* and in isolated cockroach DUM neurone cell bodies (Lapied et al., 1989).

Furthermore, the use of a K^+ channel blocker such as TEA-Cl allowed two types of responses in the development of the soma action potential to be distinguished that may be due to the appearance of a Ca^{2+}-activated K^+ current caused by a developmental increase in the inward Ca^{2+} current in the soma (Goodman and Spitzer, 1981a,b). In the identified adult locust DUM neurone DUMETi, application of TEA-Cl increased the duration of the action potential, abolished the afterhyperpolarization and caused repetitive firing (Goodman and Heitler, 1979). Finally, because Ba^{2+}, which is known to block Ca^{2+}-activated K^+ currents, evoked long-duration action potentials that were blocked by the addition of Co^{2+}, it was postulated that DUM neurone cell bodies also possessed Ca^{2+} channels.

At that time, it was assumed that efferent DUM neurones expressed voltage-dependent Na^+, Ca^{2+} and K^+ channels in the cell body membrane and were capable of generating overshooting action potentials characterized by an important afterhyperpolarizing phase that seemed to be characteristic of secretory neurones. In fact, it is now well established that most insect efferent neurones in the DUM group are neuromodulatory cells that contain and release octopamine (Morton and Evans, 1984; Stevenson and Spörhase-Eichmann, 1995; Burrows, 1996; Roeder, 1999). However, immunohistochemistry has confirmed that a number of DUM neurones also contain serotonin (Orchard et al., 1989), proctolin (Yasuyama et al., 1992), corazonin (Veenstra and Davis, 1993), myomodulin (Swales and Evans, 1994), taurine and FMRFamide (Ferber and Pflüger, 1992; Nürnberger et al., 1993; Stevenson, 1999). They innervate, according to their location in the central nervous system, different targets such as optic lobes, mushroom bodies, skeletal and visceral muscles, lateral cardiac nerve cords and also some special types of proprioceptive sense organ (Evans, 1985; Ferber and Pflüger, 1992; Orchard et al., 1993; Burrows, 1996; Sinakevitch et al., 1996; Bräunig and Eder, 1998; Bräunig, 1999).

Although the modulatory effects of DUM neurones *via* the release of octopamine have been intensively studied in insects, one of the most exciting questions, the origin of the spontaneous rhythmic activity, was surprisingly unresolved. Before the development of new experimental procedures, our understanding of DUM neurone excitability rested fundamentally in the early *in situ* intracellular electrophysiological analysis cited above, and information on the ionic mechanisms underlying this spontaneous electrical activity was lacking. DUM neurones are, however, easily distinguished from other insect neurones by their spontaneous action potentials. But what the word *spontaneous* really means is that we do not understand how this activity is generated. It is clear that spontaneous activity is not only independent of the normal operation of neuronal circuits, but occurs without circuits at all, in completely isolated cells. In other words, spontaneously active neurones, also named pacemaker neurones (Connor, 1985), generate their activity patterns endogenously, i.e. on the basis of their intrinsic membrane properties. Consequently, it is assumed that pacemaker neurones need a steady driving input to make them fire rhythmically. This driving input could be derived from constant input currents (e.g. background currents) or from adjustments of different specialized ionic conductances that give rise to a steady or time-varying inward current in the subthreshold voltage region. However, a better understanding of the ionic mechanisms promoting rhythmicity in pacemaker neuronal cells requires the use of voltage-clamp investigations. Since most of the studies on DUM neurones have been performed *in situ*, using conventional intracellular microelectrodes, potential space-clamp problems (because of the presence of extensive neuritic trees and a high series resistance) have seriously limited the characterization and detailed analysis of the ionic currents involved in the development of spontaneous electrical activity. In addition, since the demonstration of certain ionic currents depends, in large part, on the use of pharmacological blockers, and since these blockers act non-selectively on both pre- and postsynaptic membranes or act intracellularly, the characterization of the different ionic currents becomes very difficult *in situ*. This problem is reinforced by the presence of a glial blood–brain barrier (Abbott et al., 1986) surrounding the neurones and restricting the penetration of pharmacological agents.

To avoid some of these technical problems, electrophysiological analyses of insect neuronal ionic channels were extended to *in vitro* cultured or freshly dissociated embryonic or adult neurones (Usherwood et al., 1979; Beadle and Hicks, 1985; Howes et al., 1991; Smith and Howes, 1996). Furthermore, the detailed investigation of the ionic currents involved in insect neuronal electrical activity has been greatly advanced in recent years through the use of the patch-clamp technique (Hamill et al., 1981). The advent of this technique has permitted whole-cell voltage-clamp experiments to be performed on dissociated neurones using low-resistance recording pipettes and a fast solution-changing system.

The electrical activity of the dorsal unpaired median neurone: present directions

To gain further insights into the electrophysiological and firing characteristics of efferent DUM neurones, the patch-clamp technique has been developed and adapted to single adult DUM neurones isolated from the dorsal midline of the TAG of the cockroach *Periplaneta americana* (Fig. 1A,B) (Lapied et al., 1989). In the cockroach *Periplaneta americana*, DUM neurones can be seen in all ganglia of the ventral nerve cord (Dymond and Evans, 1979; Pollack et al., 1988; Tanaka and Washio, 1988; Elia and Gardner, 1990; Lapied et al., 1994; Sinakevitch et al., 1994, 1996). By using anterograde and retrograde labelling and immunocytochemistry, octopamine-like, but also co-localized octopamine- and taurine-like, immunoreactive DUM neurones have been revealed in the suboesophageal, metathoracic and abdominal ganglia (Fig. 1A) of the cockroach *Periplaneta americana* (Eckert et al., 1992; Nürnberger et al., 1993; Sinakevitch et al., 1994, 1996). Using both enzymatic treatment and mechanical dissociation of the dorsal midline of the cockroach TAG, a method has been described for the isolation of fully differentiated adult neurones (Lapied et al., 1989). In addition, it has been demonstrated that cultured cockroach or locust adult neurones (thought to be DUM neurones) can survive for several weeks and regenerate a single primary neurite that divides into two symmetrical lateral neurites with a number of fine processes radiating from the endings (Howes et al., 1991; Lapied et al., 1993; Smith and Howes, 1996). These correspond to the typical DUM neurone morphology revealed *in situ*. However, adult neurones growing in these culture systems grew extensive processes, which reduced the ability to control the electrical potential of the entire membrane surface and also hampered complete intracellular dialysis by the internal pipette solution during whole-cell patch-clamp experiments. The use of acutely isolated adult neurones (i.e. maintained in short-term culture) overcomes these weaknesses in that neurones have only a nearly pyriform cell body geometry (Fig. 1D) in which an adequate space-clamp and complete intracellular dialysis are easily attained (Lapied et al., 1989).

Cockroach DUM neurones have been cultivated in low-density culture to facilitate the study of the electrophysiological properties of individual cells. Several criteria have confirmed that adult neurones cell bodies (50–60 μm in diameter) correspond to DUM neurones. Isolated cell bodies are obtained after dissociation of the median part of the TAG. This procedure avoids contamination of the cell suspension by other neuronal cells, such as the cell bodies of giant interneurones, that are known to be situated at the periphery of the TAG (Harrow et al., 1980). The immunocytochemical procedure, using octopamine antisera (Sinakevitch et al., 1994), has revealed that, as *in situ*, isolated cell bodies with large somata show octopamine-like immunoreactivity (Fig. 1A,E). In addition, electrophysiological experiments have indicated that the electrical activity of isolated cell bodies is similar to that

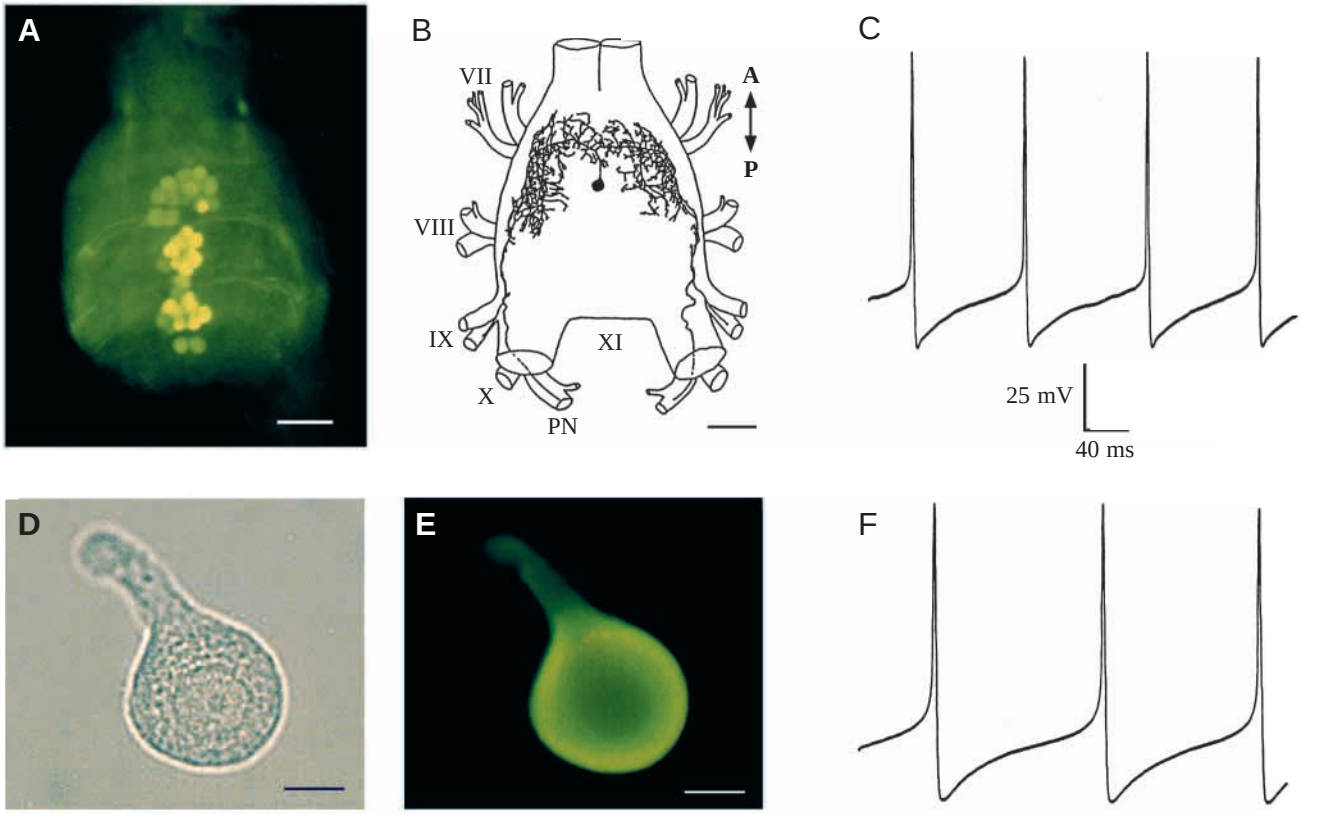

Fig. 1. Dorsal unpaired median (DUM) neurones of the terminal abdominal ganglion (TAG) of the cockroach *Periplaneta americana*. (A) Photomicrograph showing octopamine-like immunoreactive DUM neurones revealed on the dorsal surface of the TAG. Scale bar, 200 µm. (B) A dorsal-view *camera lucida* drawing of the general morphology of DUM neurones revealed by anterograde cobalt staining performed on a soma located along the midline of the TAG. Scale bar, 200 µm, A, anterior; P, posterior; VII, VIII, IX, segmental nerves; X and XI, cercal nerves; PN, phallic nerves. (C) Spontaneous overshooting action potentials recorded using an intracellular microelectrode in a DUM neurone *in situ*. The scale bar also applies to F. (D,E) Light micrographs of an isolated DUM neurone cell body maintained in short-term culture (24 h; D) and treated with a polyclonal antibody directed against octopamine (E). Scale bars, 25 µm. (F) Spontaneous electrical activity recorded using the patch-clamp technique (whole-cell recording configuration) in a single DUM neurone cell body.

recorded *in situ* from the neurones located along the dorsal midline of the TAG (Lapied et al., 1989; Figs 1C,F, 2A). This electrical activity is fundamentally different from that recorded, for instance, in TAG dorsal paired median neurones, which are present among the population of DUM neurones but are known to generate Ca^{2+}-dependent somatic plateau action potentials (Amat et al., 1998). On the basis of these results, the use of dissociated adult DUM neurones combined with the patch-clamp technique confirms (i) that isolated adult DUM neurone cell bodies are capable of generating spontaneous electrical activity, like pacemaker neurones (Figs 1F, 2A), and (ii) that isolated DUM neurones retain the favourable characteristics of acutely isolated neurones for both whole-cell current- and voltage-clamp experiments without detrimental changes in their electrophysiological properties. Using this new experimental approach, voltage-clamp investigations of the ionic currents underlying DUM neurone electrical activity have been greatly advanced in recent years.

This review focuses primarily on the biophysical and pharmacological properties, but also on the specific functional roles, of the ionic channels involved in the generation of the spontaneous activity of efferent DUM neurones.

Properties and regulation of voltage-dependent ionic channels

Ionic channels activated upon depolarization

Na^+ channels: properties and physiological implications

The existence of voltage-dependent Na^+ channels was suspected in efferent DUM neurones (Jego et al., 1970; Goodman and Heitler, 1979; Goodman and Spitzer, 1981a,b; Cook and Orchard, 1993). Their presence was confirmed in cockroach adult DUM neurones, isolated from the abdominal ganglia (i.e. abdominal ganglion 5, A5, and TAG), using complementary approaches such as the patch-clamp technique (whole-cell recording configuration) (Lapied et al., 1989, 1990b; Wicher and Penzlin, 1998) and immunocytochemistry (Amat et al., 1998). Under current-clamp condition, it was reported that action potentials recorded both *in situ* and from isolated cell bodies are completely abolished by extracellularly

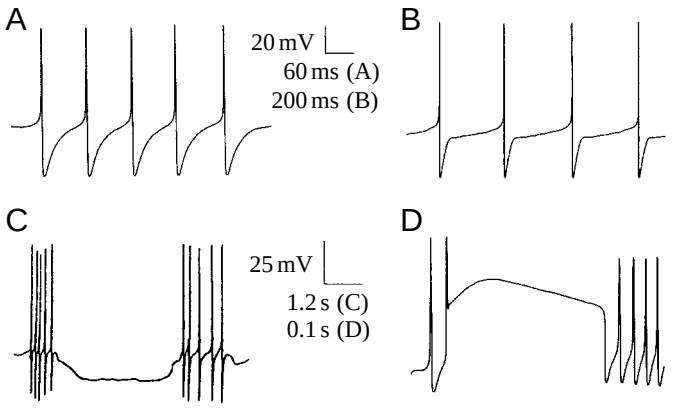

Fig. 2. Different firing patterns of dorsal unpaired median (DUM) neurones recorded using the patch-clamp technique (whole-cell recording configuration). (A) Typical example of a regularly spiking DUM neurone cell body. (B) Some DUM neurones can fire repetitive action potentials at regular intervals but at a very low firing frequency. In this case, these DUM neurones are insensitive to low concentrations of nickel chloride and to high external Ca^{2+} concentrations, suggesting that these DUM neurone cell bodies do not express low-voltage-activated (LVA) Ca^{2+} channels (see text for details). (C) DUM neurone cell bodies can discharge groups of action potentials separated by slow hyperpolarized potentials. This transition from beating to bursting electrical activity is conditional upon changes in the behaviour of resting Na^+ channels induced by scorpion α-toxins. (D) When inactivation of the voltage-dependent Na^+ current of the DUM neurone is slowed down by treatment with scorpion α-toxins, the falling phase of the action potential develops a prolonged shoulder resulting in a plateau action potential.

applied tetrodotoxin (TTX) or saxitoxin (Lapied et al., 1989). Thus, it appears that Na^+ alone is sufficient for the generation of the soma spike in cockroach TAG DUM neurones, in contrast to locust metathoracic DUM neurones (i.e. DUMETi) in which both Na^+ and Ca^{2+} are necessary (Goodman and Heitler, 1979; Goodman and Spitzer, 1981a). However, it was demonstrated in DUMETi neurones that the Ca^{2+}/Na^+-dependent soma spike could be converted into a Na^+-dependent soma spike following axotomy or treatment with colchicine (Goodman and Heitler, 1979). In this case, addition of Co^{2+} did not affect the action potential. This conversion of somatic action potentials from being sensitive to Co^{2+} to being resistant to Co^{2+} was interpreted to be the result of an increase in the number of active Na^+ channels, either by addition of new channels or by redistribution of existing channels (Goodman and Heitler, 1979; Titmus and Faber, 1990). In cockroach TAG DUM neurones, the Na^+-dependent overshooting action potentials are not induced by axotomy and deafferentation since Na^+-free saline or TTX completely blocks soma spikes both *in situ* and in isolated cells (Jego et al., 1970; Lapied et al., 1989). However, axotomy and deafferentation appear to enhance somata excitability. The studies on TAG DUM neurones have demonstrated that axotomy and deafferentation induce an increase in the maximum peak current amplitude without any modification of the kinetics (activation and inactivation) of the Na^+ current. By using protein synthesis inhibitors (cycloheximide and actinomycin D), it has been demonstrated that the evolution of the peak current amplitude observed in isolated DUM neurones was due to *de novo* synthesis of Na^+ channels (Tribut et al., 1991).

Voltage-dependent Na^+ channels in TAG DUM neurone cell bodies have been localized by immunocytochemistry using antibodies directed against a synthetic peptide corresponding to the highly conserved SP19 segment of the rat brain type I voltage-dependent Na^+ channel α-subunit (Amat et al., 1998). Light microscopy has revealed that voltage-dependent Na^+ channels are heterogeneously distributed in TAG DUM neurone somata. The intensity of staining gradually decreases from the basal region to the apical pole, where no significant SP19 immunoreactivity is observed (Amat et al., 1998). These results indicate that voltage-dependent Na^+ channels are mainly located close to the initial segment of the soma. This confirms the localization of one of the four regions of spike initiation previously proposed in DUMETi (Heitler and Goodman, 1978).

Voltage-clamp experiments provide further insight into the biophysical properties of the cockroach DUM neurone inward Na^+ current. When the concentration of extracellular Na^+ is varied, the reversal potential of the inward current shifts as expected from the Nernst equation, confirming that the inward current is highly selective for Na^+ (Lapied et al., 1990b). The inward Na^+ current is very sensitive to TTX. The concentration for half-maximal current inhibition (IC_{50}) varies somewhat among different preparations between 2 nmol l^{-1} (B. Lapied, unpublished observations) and 10.5 nmol l^{-1} (Wicher and Penzlin, 1998). This current is also partially blocked by external veratridine, ω-agatoxin and ω-conotoxin MVIIC, which reduces the time constant of inactivation (Wicher and Penzlin, 1998). The voltage-dependent Na^+ current activates at potentials more positive than −40 mV (e.g. −35 mV), corresponding to the threshold of spontaneous action potentials (Lapied et al., 1989), and reaches a current maximum at approximately −10 mV (Lapied et al., 1990b; Wicher and Penzlin, 1998). The Na^+ current exhibits sigmoidal voltage-dependent activation kinetics, and both activation and inactivation of the current become faster with strong depolarization. The voltage-dependence of steady-state inactivation and activation indicates that the Na^+ channels are half-inactivated at approximately −40 mV and half-activated near −25 mV. The inactivation and activation curves cross at approximately −30 mV, resulting in a region of activation/inactivation overlap that extends from approximately −75 to +10 mV. This suggests the existence of a large steady-state window current, indicating that some of the channels activated are never inactivated in this potential range (Lapied et al., 1990b). It should be noted that, in addition to this transient inward current, an unexpected maintained voltage-dependent Na^+ current has also been characterized in TAG DUM neurones (Lapied et al., 1990b). This Na^+ current, also sensitive to TTX, is biophysically isolated from the other current by its negative threshold for activation (approximately

–70 mV). This suggests the existence of two distinct voltage-dependent Na^+ currents in TAG DUM neurones. However no more information is available about the precise biophysical properties and physiological role of this low-threshold current in DUM neurone electrical activity.

As in many excitable cells, the activation of the TAG DUM neurone voltage-dependent Na^+ current controls the rising phase of spontaneous action potentials (Lapied et al., 1989), whereas the inactivation of the current is involved in the declining phase. This is particularly important since it has been demonstrated that neurotoxic compounds such as scorpion toxins, which are known to affect activation and particularly inactivation processes of insect voltage-dependent Na^+ currents (Pelhate et al., 1998), transform, in TAG DUM neurones, a short-duration action potential (approximately 2 ms in duration, Lapied et al., 1989) into a plateau action potential (0.5–20 s in duration; Fig. 2D; Stankiewicz et al., 1996). Such plateau action potentials could enhance the internal Ca^{2+} concentration and thereby influence the somatic neuronal function of DUM neurones.

K^+ channels: properties and physiological implications

The K^+ channels in DUM neurones were first revealed *in situ* under current-clamp conditions (for references, see Introduction). In isolated cockroach DUM neurones, TEA-Cl reduced the delayed outward rectification, as demonstrated in other DUM neurones (Goodman and Spitzer, 1981a,b; Washio and Tanaka, 1992), prolonged the falling phase of action potentials and suppressed the afterhyperpolarization (Lapied et al., 1989). These effects, mimicked by treatment with a Ca^{2+}-free saline, led to the suggestion that a Ca^{2+}-activated K^+ current was involved in both the repolarization and afterhyperpolarization of the action potentials, as previously reported in grasshopper DUM neurones (Goodman and Spitzer, 1981a). In fact, the first evidence for the presence of a Ca^{2+}-activated K^+ current came from experiments performed under voltage-clamp conditions in cockroach metathoracic DUM neurones (Thomas, 1984). This study demonstrated that ionophoretic injection of Ca^{2+} elicited an outward current carried by K^+. The outward current showed an N-shaped current–voltage relationship, as typically observed for Ca^{2+}-mediated K^+ currents, and was blocked by lanthanum and the organic Ca^{2+} antagonist D-600.

Further electrophysiological and pharmacological investigations performed under voltage-clamp conditions revealed an unexpected diversity of K^+ channels underlying the electrical activity of the DUM neurone. Earlier studies performed using the patch-clamp technique (cell-attached configuration) had revealed the existence of at least three types of outward current in cockroach metathoracic DUM neurones distinguished by their unitary conductance (11, 34 and 110 pS) (Dunbar and Pitman, 1985). Although the ionic properties of these channels was not determined, two of these channels (with conductances of 11 and 34 pS) were shown to conduct K^+.

Whole-cell voltage-clamp experiments have allowed the identification of at least five distinct types of K^+ channel in isolated cockroach DUM neurones (Grolleau and Lapied, 1994, 1995a; Wicher et al., 1994). These K^+ channels can be classified into two groups, the first type activated by both voltage and intracellular ions and the second type only by voltage. Two distinct K^+ currents activated by intracellular ions have been characterized in DUM neurones. The Na^+-activated K^+ current is one of the most unexpected currents (Dryer, 1994) since, usually, K^+ currents are directly studied electrophysiologically in the presence of Na^+ channel blockers. However, it has been demonstrated in both the TAG DUM neurone (Grolleau and Lapied, 1994) and the fifth abdominal ganglion (A5) DUMIa neurone (Gundel et al., 1996) that a fraction of the global outward current was sensitive to TTX. The tail current analysis, used to determine the ionic selectivity of this outward current, has confirmed that it is carried by K^+. In fact, further electrophysiological experiments demonstrated that the disappearance of this component is related to the suppression of the inward Na^+ current by TTX or by Na^+-free saline (Grolleau and Lapied, 1994). The progressive activation of the Na^+-activated K^+ current is well correlated with the activation of the inward Na^+ current, indicating that this K^+ current is activated by the entry of Na^+ into the DUM neurone. Because there is no selective blocker of Na^+-activated K^+ currents available, the physiological significance of such currents in DUM neurone electrical activity is unknown. Nevertheless, it is suggested that this current could limit the action potential duration by increasing the rate of repolarization of the action potential (Grolleau and Lapied, 1994).

The second type of K^+ channel activated by intracellular ions corresponds to the most commonly found Ca^{2+}-activated K^+ channels (Rudy, 1988). Electrophysiological experiments performed on cockroach DUM neurones have indicated that such currents are also voltage-dependent (Thomas, 1984; Wicher et al., 1994; Grolleau and Lapied, 1995a; Achenbach et al., 1997). The Ca^{2+}-activated K^+ current of the TAG DUM neurone activates rapidly at potentials more positive than –50 mV and then declines in two phases. Both the transient and late components of the current are sensitive to extracellular TEA-Cl and to different scorpion toxins known to affect Ca^{2+}-activated K^+ currents (Garcia et al., 1991) (Table 1). The existence of such K^+ currents has also been confirmed by preventing Ca^{2+} permeation of the membrane using classical inorganic Ca^{2+} channel blockers such as cadmium chloride and nickel chloride (Wicher et al., 1994; Grolleau and Lapied, 1995a). The biphasic aspect of the Ca^{2+}-activated K^+ current, together with the different steady-state holding potential sensitivity of the two components, led to the suggestion of the existence of two separate Ca^{2+}-activated K^+ currents in TAG DUM neurones. However, this hypothesis has never been confirmed since none of the specific Ca^{2+}-activated K^+ current blockers tested allows discrimination between the fast transient and late components of the current.

Ca^{2+}-activated K^+ currents have different physiological implications for the electrical activity of the DUM neurone. The prolongation of the falling phase of action potentials

Table 1. *Voltage-activated ionic currents in cockroach dorsal unpaired median neurones*

Ionic current	Activation threshold (mV)	Inactivation	Pharmacology	Physiological role	Reference
Ionic currents activated upon depolarization					
I_{Na} (TAG and A5)	–35	Yes	TTX, saxitoxin; partially blocked by ω-conotoxin MVIIC ω-agatoxin, veratridine, scorpion α-toxin	Action potential depolarization	Lapied et al. (1990b) Wicher and Penzlin (1998) Pelhate et al. (1998)
$I_{Na,m}$ (TAG)	–75	Slowly or none	Saxitoxin	?	Lapied et al. (1990b)
$I_{K,Ca}$ (metathoracic)	–25, –30	None	Lanthanum, D-600	Repolarization?	Thomas (1984)
$I_{K,Ca}$ (TAG and A5)	–40	Two phases	TEA-Cl, $CdCl_2$, charybdotoxin, iberiotoxin	Repolarization, after hyperpolarization	Wicher et al. (1994) Grolleau and Lapied (1995a) Achenbach et al. (1997)
$I_{K,Na}$ (TAG)	–35	Yes	All K^+ blockers	Limitation of action potential duration?	Grolleau and Lapied (1994)
$I_{K,Na}$ (A5)	?	?	?	?	Gundel et al. (1996)
$I_{K,A}$ (TAG)	–65	Yes	4-Aminopyridine	Regulation of firing frequency	Grolleau and Lapied (1995a)
$I_{K,DR}$	–45, –50	None	TEA-Cl (>30 mmol l^{-1}), internal CsCl	Part of repolarization	Grolleau and Lapied (1995a)
I_{Ca} tLVA (TAG)	–70	Yes, voltage-dependent	<100 µmol l^{-1} $NiCl_2$, $CdCl_2$	Initial part of the predepolarization	Grolleau and Lapied (1996)
I_{Ca} mLVA (TAG)	–60	Slowly or none, Ca^{2+}- and voltage-dependent	>100 µmol l^{-1} $NiCl_2$, $CdCl_2$	Last two-thirds of the predepolarization	Grolleau and Lapied (1996)
I_{Ca} M-LVA (A5)	–50	Incomplete	$NiCl_2$, $CdCl_2$, ω-conotoxin MVIIC, ω-agatoxin IVA	Control of repolarization and afterhyperpolarization *via* $I_{K,Ca}$	Wicher and Penzlin (1997)
I_{Ca} HVA (TAG and A5)	–30, –40	Incomplete, voltage-dependent	$CdCl_2$, reduced by verapamil, diltiazem, ω-conotoxin GVIA	Control of repolarization and afterhyperpolarization *via* $I_{K,Ca}$	Wicher and Penzlin (1994, 1997) Grolleau and Lapied (1996)
Ionic currents activated upon hyperpolarization					
$I_{K,IR}$	–75, –80	None	TEA-Cl, $BaCl_2$, external CsCl	Inward rectification	Raymond and Lapied (1999)
$I_{Cl,Ca}$	–60	None	Internal SITS, DIDS, $ZnCl_2$, internal BAPTA, Ca^{2+}-free saline	Limitation of excessive hyperpolarization	Raymond and Lapied (1999)

TAG, terminal abdominal ganglion; A5, fifth abdominal ganglion; LVA, low-voltage-activated; mLVA, maintained LVA Ca^{2+} current; M-LVA, mid/low voltage-activated Ca^{2+} current; tLVA, transient LVA Ca^{2+} current; HVA, high-voltage-activated; TTX, tetrodotoxin.

associated with a suppression of the afterhyperpolarization observed in the presence of Ca^{2+}-activated K^+ channel blockers, Ca^{2+}-free saline or inorganic voltage-dependent Ca^{2+} channel blockers (Lapied et al., 1989) indicates that these currents play an important role in spike repolarization (i.e. in the regulation of action potential duration) and in the regulation of the repetitive discharge frequency of the DUM neurone. This has been confirmed by recent electrophysiological investigations indicating that the electrical pattern of DUM neurone discharges is influenced by both up- and down-modulation of Ca^{2+}-activated K^+ currents induced by neurohormone D (Glp-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2; a member of the family of adipokinetic hormones; Penzlin, 1989) and octopamine *via* an effect on voltage-dependent Ca^{2+} currents (Wicher and Penzlin, 1994; Wicher et al., 1994; Achenbach et al., 1997).

Besides K^+ currents activated by intracellular ions, two additional K^+ currents activated by voltage have also been characterized in cockroach DUM neurones (Table 1). Using biophysical and pharmacological approaches, it has been possible to dissect the ion-independent K^+ currents into two further voltage-dependent K^+ currents identified as an A-like current and a delayed outward rectifier K^+ current (Grolleau and Lapied, 1995a). The A-like current, also termed the fast transient K^+ current, was first described in invertebrates and in particular in gastropod neural somata (Connor and Stevens, 1971). Because the A-like current is selectively blocked by 4-aminopyridine (4-AP) in DUM neurones, it has been isolated by subtracting the residual current after treatment with 4-AP from the global voltage-dependent K^+ current. This current activates at depolarizing potentials more positive than −70 mV and exhibits rapid time-dependent activation and inactivation. Half-maximal steady-state inactivation occurs at approximately −65 mV and, unlike the classical A-current of other neurones (Hille, 1992), this current inactivates with complex inactivation kinetics (Grolleau and Lapied, 1995a). Steady-state activation and inactivation curves cross at approximately −53 mV, resulting in a region of activation/inactivation overlap that extends from approximately −70 to −30 mV. This indicates that these channels conduct only within a window of negative potentials.

As previously reported in other vertebrate and invertebrate pacemaker neurones, the A current plays a fundamental role in the modulation of action potential frequency (Rudy, 1988; Hille, 1992). In cockroach DUM neurones, the physiological implication of the A-like current has been demonstrated using 4-AP under current-clamp conditions (Grolleau and Lapied, 1995a). These results indicate that the A-like current is not involved in the repolarization and afterhyperpolarization of the action potentials. In contrast, the 4-AP-induced increase in action potential frequency indicates that the A-like current, which is activated when the membrane is hyperpolarized beyond the resting level, regulates the behaviour of the DUM neurone repetitive discharge frequency.

The final voltage-dependent K^+ current characterized in DUM neurones is the delayed outward K^+ current described in both excitable and non-excitable preparations (Rudy, 1988; Pelhate et al., 1990; Hille, 1992). As in other neuronal preparations, this K^+ current activates slowly and follows a sigmoidal time course to reach its steady-state value. This current shows no inactivation during the course of a maintained depolarization. It activates at potentials more positive than −50 mV and is completely blocked by a high external TEA-Cl concentration (Table 1). On the basis of its time course of activation, it is postulated that the delayed outward K^+ current contributes to the repolarizing phase of the action potential (Grolleau and Lapied, 1995a).

Ca^{2+} channels: properties and physiological implications

Ca^{2+} influx through voltage-activated Ca^{2+} channels obviously plays a central role in shaping spontaneous neuronal electrical activity since internal [Ca^{2+}] itself controls the activation of other ionic channels (e.g. Ca^{2+}-activated K^+ channels) but also controls the inactivation of the Ca^{2+} channel. This last point is of particular interest since it effectively regulates the influx of Ca^{2+}. Furthermore, Ca^{2+} entry into nerve cell bodies through Ca^{2+} channels is generally accepted as an important step in the sequence of events underlying the synthesis and release of secretory products. Consequently, in the context of the DUM neurone, electrophysiological and pharmacological investigations of the voltage-dependent Ca^{2+} channels represent one of the most exciting challenges in the our search for information about both the generation and regulation of spontaneous electrical activity. Previous current-clamp investigations indicated the existence of voltage-activated Ca^{2+} channels in the soma membrane of DUM neurones (Goodman and Heitler, 1979; Bindokas and Adams, 1989; Lapied et al., 1989). Under normal conditions, overshooting action potentials recorded in adult DUM neurone somata resulted from a combination of voltage-activated Na^+, Ca^{2+} and K^+ currents. Under appropriate conditions (in the presence of Na^+-free saline and/or K^+ channel blockers), DUM neurones could generate long-duration Ca^{2+} spikes that were blocked by the addition of Co^{2+} or ω-agatoxin-I (isolated from the venom of the spider *Agelenopsis aperta*; Goodman and Heitler, 1979; Bindokas and Adams, 1989). Furthermore, the three effects on action potentials (i.e. an increase in action potential duration, a reduction in the afterhyperpolarization and a positive shift of the threshold of the action potential) observed in the presence of different inorganic Ca^{2+} channels blockers (Lapied et al., 1989) also indicated that DUM neurones contained somatic voltage-activated Ca^{2+} channels.

The first indication of the presence of different types and subtypes of voltage-activated Ca^{2+} channel in DUM neurone cell bodies came from electrophysiological and pharmacological studies performed under voltage-clamp conditions (Wicher and Penzlin, 1994, 1997; Grolleau and Lapied, 1996; Grolleau et al., 1996; Achenbach et al., 1997). Voltage-activated Ca^{2+} channels have been classified using a variety of criteria including ionic selectivity, voltage and ionic sensitivity, pharmacological profile and sensitivity to physiological ligands (e.g. nicotine, octopamine,

neurohormone D). Accordingly, it has been possible to define several distinct classes of Ca^{2+} channel including most notably low-voltage-activated (LVA) and high-voltage-activated (HVA) Ca^{2+} channels (Table 1). However, from these studies, it is clear that subtypes of these channels, such as maintained LVA Ca^{2+} channels (Grolleau and Lapied, 1996) and M-LVA Ca^{2+} channels (Wicher and Penzlin, 1997), also exist in DUM neurone somata, demonstrating the difficulty in establishing a classical classification of voltage-activated Ca^{2+} channels. Nevertheless, both specific and/or selective pharmacological agonists (e.g. BAY K 8644) and antagonists (organic and inorganic Ca^{2+} channel blockers, peptide toxins isolated from the *Conus* and *Agelenopsis* venoms) have provided a very useful way of classifying and sub-classifying DUM neurone voltage-dependent Ca^{2+} channels (see Table 1).

In cockroach DUM neurones, the LVA and HVA Ca^{2+} currents differ from each other on the basis of their sensitivity to different Ca^{2+} channel blockers (see Table 1), but also in their voltage-dependence of activation and inactivation. Activation at very negative membrane potentials (between −70 to −60 mV), measured at physiological internal Ca^{2+} concentration (Grolleau and Lapied, 1996), is the main characteristic of the LVA Ca^{2+} currents in neuronal preparations (for a review, see Kostyuk, 1999). In cockroach TAG DUM neurones, the global LVA Ca^{2+} current has been further dissociated by means of the sensitivity to nickel chloride, the time constant of deactivation and the kinetics of inactivation into transient LVA (tLVA) and maintained LVA (mLVA) Ca^{2+} currents (Grolleau and Lapied, 1996). The tLVA current resembles the classical LVA Ca^{2+} current described previously (Kostyuk, 1999). This current activates at a potential more positive than −80 mV and inactivates completely during a prolonged depolarizing pulse. It is completely blocked by a relatively low concentration of nickel (<100 μmol l^{-1}), and the kinetics of its inactivation is independent of Ca^{2+} influx through the channel. The potential at which half the tLVA channels are inactivated is approximately −60 mV. In contrast, the mLVA Ca^{2+} current, characterized for the first time in DUM neurones (Grolleau and Lapied, 1995b) and confirmed later in other neuronal preparations (Kostyuk, 1999), corresponds to a new subtype of LVA Ca^{2+} current. This mLVA current is easily distinguished from the tLVA current by its very slow kinetics of inactivation (it does not show complete inactivation during the course of the depolarizing pulse), by its activation threshold (10 mV more positive than that of the tLVA current), by its pharmacological profile (see Table 1) and by its Ca^{2+}-sensitive inactivation. The mLVA current becomes completely inactivated by any means that increases or reduces the intracellular Ca^{2+} concentration (Grolleau and Lapied, 1996). Furthermore, it has been demonstrated that the voltage-dependence of inactivation displays an unexpected U-shaped curve. This is consistent with the existence of a complex inactivation mechanism controlled by both voltage and intracellular Ca^{2+} concentration. This property, unusual for an LVA Ca^{2+} current, underlies the unique physiological function of this current in the firing pattern of DUM neurones *via* the intracellular Ca^{2+} concentration (see below).

Beside these LVA Ca^{2+} currents, a mid/low-voltage-activated Ca^{2+} current (M-LVA, Wicher and Penzlin, 1997) and an HVA Ca^{2+} current (Grolleau and Lapied, 1996; Wicher and Penzlin, 1997) have also been characterized in both cockroach TAG neurones and fifth abdominal ganglion DUM neurones. The M-LVA Ca^{2+} current activates in the mid-voltage range (e.g. −50 mV). Although the study of the voltage dependence of the steady-state inactivation indicates that the inactivation process is voltage-dependent and Ca^{2+}-independent, the M-LVA Ca^{2+} current displays a time-dependent inactivation that is Ca^{2+}-dependent (Wicher and Penzlin, 1997). The time-dependent decay is strongly reduced when Ba^{2+}, known to pass through Ca^{2+} channels, is substituted for Ca^{2+}. As indicated in Table 1, M-LVA Ca^{2+} currents are blocked by inorganic Ca^{2+} channel blockers (IC_{50} values of approximately 20 μmol l^{-1} for nickel chloride and 10 μmol l^{-1} for cadmium chloride). Peptide toxins such as ω-conotoxin MVIIC (at 1 μmol l^{-1}) and ω-agatoxin IVA (at 50 nmol l^{-1}) also block the M-LVA Ca^{2+} currents. In contrast, M-LVA Ca^{2+} currents are enhanced by octopamine (1–10 μmol l^{-1}). This potentiation is mimicked by a membrane-permeant cyclic AMP analogue (8-bromo-cyclic AMP), indicating a possible modulation of M-LVA currents by octopamine *via* cyclic AMP (Achenbach et al., 1997). Because M-LVA Ca^{2+} currents are not affected by amiloride or flunarizine, which are known to block vertebrate neuronal LVA Ca^{2+} currents, it has been postulated that the DUM neurone M-LVA Ca^{2+} current has no similarity with classical LVA Ca^{2+} currents.

The last type of Ca^{2+} current characterized in DUM neurones corresponds to the HVA current (Grolleau and Lapied, 1996; Wicher and Penzlin, 1997). At physiological Ca^{2+} concentrations, HVA currents activate at potentials more positive than −40 mV. During the depolarizing pulse, the current reaches a peak and then declines as channels inactivate. However, on return to the initial membrane potential, open channels close and the current deactivates, indicating the existence of incomplete inactivation. The value for half-maximal steady-state inactivation is estimated to be approximately −45 mV. DUM neurone HVA currents are carried by Ca^{2+} or Ba^{2+} through Ca^{2+} channels and are blocked by cadmium and nickel (estimated IC_{50} values are 5 μmol l^{-1} for cadmium chloride and 40 μmol l^{-1} for nickel chloride). HVA currents are reduced by verapamil and diltiazem (Wicher and Penzlin, 1997), which are regarded as non-selective blockers (Triggle, 1999), and by octopamine (100 μmol l^{-1}) (Achenbach et al., 1997). In contrast, HVA Ca^{2+} currents are not affected by dihydropyridines such as nifedipine and Ca^{2+} agonists such as the racemic BAY K 8644, which is often used to identify dihydropyridine-sensitive Ca^{2+} channels. Arthropod and molluscan toxins of high selectivity have proved to be crucial for the identification of Ca^{2+} channels. The neuropeptide ω-conotoxin GVIA purified from the venom of the marine snail *Conus geographus* is the toxin used most

widely to study neuronal HVA Ca^{2+} channels. In DUM neurones, HVA currents are also sensitive to ω-conotoxin GVIA in the concentration range 0.1–1 μmol l^{-1} (F. Grolleau, unpublished observations; Wicher and Penzlin, 1997).

Although the functional significance of both M-LVA and HVA Ca^{2+} currents has not been directly assessed, it has been postulated that these Ca^{2+} currents play a key role in the control of the afterhyperpolarizing phase *via* the modulation of the Ca^{2+}-activated K^+ current (Lapied et al., 1989). This is of particular interest since we have already indicated that M-LVA and HVA Ca^{2+} currents are modulated by physiological modulators such as neurohormone D and octopamine (Wicher and Penzlin, 1994; Achenbach et al., 1997), which thereby influence the shape of the action potentials *via* the Ca^{2+}-activated K^+ current. In contrast, it has been demonstrated that the two distinct LVA Ca^{2+} channels have specialized functions in the generation of spontaneous electrical activity (Grolleau and Lapied, 1996). In pacemaker neurones, spontaneous activity results from a typical phase of their action potential, the slow predepolarization. During this phase, the membrane slowly depolarizes following the termination of an action potential until the threshold for a new action potential is reached. Because LVA Ca^{2+} currents are deinactivated when the membrane is hyperpolarized beyond the resting level, they are one of the most suitable candidates to promote this predepolarization. In spontaneously active TAG DUM neurones, it has been demonstrated pharmacologically that LVA Ca^{2+} currents contribute to the predepolarizing phase (Grolleau and Lapied, 1996). The transient LVA currents are essential for initiating the first part of the predepolarization, whereas mLVA Ca^{2+} currents plays a determinant role in the last two-thirds of the pacemaker potential. These mLVA Ca^{2+} currents, which are controlled by intracellular Ca^{2+} concentration, influence both the shape of the predepolarization and also the spontaneous firing frequency since complete inactivation of this current by changes in internal Ca^{2+} concentration induces an important reduction in the frequency of spontaneous action potentials. This property has been confirmed using cholinergic ligands such as nicotine. Although nicotine is known to depolarize the TAG DUM neurone membrane potential (Lapied et al., 1990a), it reduces DUM neurone firing frequency by modulating the mLVA Ca^{2+} current *via* an increase in intracellular Ca^{2+} concentration (Grolleau et al., 1996). It is interesting to note that some adult DUM neurones that do not express LVA Ca^{2+} currents (F. Grolleau and B. Lapied, personal observations) display a different firing pattern with a pronounced afterhyperpolarization followed by a very slow predepolarizing phase (Fig. 2B). In this case, they are also capable of generating spontaneous action potential, but at a lower frequency.

Ionic channels activated upon hyperpolarization

Inward rectifier K^+ channel: properties and physiological implications

Another type of voltage-dependent current, the hyperpolarization-activated inward current, is also heavily involved in regulating spontaneous neuronal electrical activity. Among these inward currents, the hyperpolarization-activated inward K^+ current ($I_{K,IR}$) and the cationic (Na^+/K^+) inward current (I_H) are the best-studied currents in the responsiveness and pattern activity of neurones (Constanti and Galvan, 1983; Pape, 1996; Lüthi and McCormick, 1998). In spontaneously active TAG DUM neurones, two distinct types of hyperpolarization-acivated inward K^+ and Ca^{2+}-sensitive Cl^- current have been described, whereas I_H, which is known to play a key role in neuronal pacemaker activity (Lüthi and McCormick, 1998), has not been identified.

The electrophysiological properties of the inward rectifier K^+ current characterized in TAG DUM neurones (Raymond and Lapied, 1999) closely resemble those of inward rectifier K^+ currents in many other cells (Rudy, 1988; Hille, 1992). This inward K^+ current activates at potentials near −80 mV, is half-activated at −109 mV and does not show time-dependent inactivation in normal saline solution. This current is permeable to K^+ since the reversal potential follows the K^+ equilibrium potential when the extracellular K^+ concentration is increased. It is not dependent on external Na^+ concentration and is sensitive to CsCl, $BaCl_2$ and TEA-Cl, but at high concentration (see Table 1; Raymond and Lapied, 1999). Under current-clamp conditions, injection of a long hyperpolarizing current pulse into the cell body induces a time-dependent rectification seen as a depolarizing sag in the electrotonic potential. Although it has been demonstrated that the inward K^+ current accounts for inward rectification of TAG DUM neurones, the precise physiological role of this current in the regulation of the electrical activity of the DUM neurone is not clear. The very negative potential range in which the current is activated indicates that it does not affect DUM neurone excitability at or near the resting membrane potential. However, it may act as an important depolarizing mechanism that prevents TAG DUM neurones from becoming unresponsive when they are excessively hyperpolarized.

Ca^{2+}-sensitive Cl^- channel: properties and physiological implications

Voltage-gated Cl^- channels have been regularly detected in both lower and higher animals. Among them, hyperpolarization-activated inward Cl^- currents and particularly inward Ca^{2+}-sensitive Cl^- currents have been less studied. Hyperpolarization-activated inward Cl^- currents were first characterized in *Aplysia* neurones (Chesnoy-Marchais, 1990). However, in some cases, these currents were only revealed after Cl^- loading of the cell using microelectrodes containing a high concentration of Cl^-, indicating that these currents were not active under normal physiological conditions. In TAG DUM neurones, hyperpolarizing voltage pulses elicit a slowly activated inward Cl^- current that does not require Cl^- loading for its detection (Raymond and Lapied, 1999). This inward current is activated over a range of potential 20 mV more positive than the inward rectifier K^+ currents (half-activation at −99 mV) and does not inactivate

during the hyperpolarizing voltage pulse. The estimated reversal potential is very close to the calculated equilibrium Cl^- potential and is not affected by changes in external K^+ and Na^+ concentrations. Intracellularly applied 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) and 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) and external application of $ZnCl_2$ selectively inhibit the inward Cl^- current. One of the most interesting features of this hyperpolarization-activated inward Cl^- current is its sensitivity to internal Ca^{2+} concentration. Ca^{2+}-free saline or internal application of a high concentration of 1,2-bis-(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetate (BAPTA), known to be an efficient Ca^{2+} chelator, completely blocks the Cl^- current, indicating that an influx of extracellular Ca^{2+} is required for activation of this current. This observation was not easy to understand: how could the influx of Ca^{2+} activate a hyperpolarization-activated inward current? An interesting hypothesis, however, has been proposed, on the basis of results obtained previously in the same preparation and in the fifth abdominal ganglion DUM neurones (Wicher et al., 1994; Heine and Wicher, 1998). These authors have clearly characterized a voltage-independent Ca^{2+} current functional at the resting membrane potential and flowing at hyperpolarized potentials (see below). Because increasing hyperpolarization (more negative than −60 mV) increases the size of this resting Ca^{2+} current and, consequently, elevates intracellular Ca^{2+} concentration (Heine and Wicher, 1998), it is tempting to suggest a contribution from such a current to the activation of the hyperpolarization-activated Ca^{2+}-sensitive Cl^- current.

The functional significance of this Cl^- current in the regulation of DUM neurone excitability has only been suggested. Because the intracellular Cl^- concentration is maintained at a relatively low level by active outwardly directed furosemide-sensitive Cl^-/K^+ cotransport (Dubreil et al., 1995), the inward Ca^{2+}-sensitive Cl^- current is functional in pacemaker DUM neurones under physiological conditions. On the basis of its activation threshold (−60 mV), this inward current could help to maintain spontaneous electrical activity by limiting excessive hyperpolarization induced, for example, by endogenously released inhibitory neurotransmitters such as γ-aminobutyric acid (GABA; Dubreil et al., 1994).

Properties of resting ionic channels

Resting ionic channels

Another less well-studied population of ionic channels, termed the resting (background) ionic channels, also plays a key role in the regulation of DUM neurone excitability. Previous electrophysiological investigations performed on cockroach and locust DUM neurones suggested and/or demonstrated the participation of K^+, Na^+ and Ca^{2+} resting currents in the maintenance of the resting membrane potential (Jego et al., 1970; Goodman and Spitzer, 1981a; Lapied et al., 1989). The relatively positive value of the resting membrane potential of DUM neurones (between −45 and −60 mV, i.e. above the Cl^- and K^+ equilibrium potentials) indicates that the resting membrane potential is not only due to K^+ resting currents but also suggests the involvement of, at least, Na^+ and Ca^{2+} resting currents (Goodman and Spitzer, 1981a; Lapied et al., 1989; Wicher and Reuter, 1993).

Ca^{2+} resting current

The first experimental evidence revealing the existence of a Ca^{2+} resting current in DUM neurones came from experiments performed with neurohormone D, which elevated intracellular Ca^{2+} concentration *via* the potentiation of a putative Ca^{2+} resting current (Wicher and Reuter, 1993). Voltage-clamp experiments performed later on both cockroach TAG and fifth abdominal ganglion DUM neurones have confirmed the participation of such currents in the regulation of firing properties (Wicher et al., 1994; Heine and Wicher, 1998). The resting Ca^{2+} current is voltage-independent and flows at the resting membrane potential. The current–voltage relationship indicates that the size of the current increases when the membrane potential is hyperpolarized to levels more negative than −50 mV. There is no indication of a contribution from Na^+, K^+ or Cl^- to this current (Wicher et al., 1994), indicating that it is predominantly permeable to Ca^{2+}. The resting current is strongly reduced by cadmium chloride (0.1 mmol l^{-1}) and when external Ca^{2+} concentration is reduced from 5 mmol l^{-1} (normal conditions) to 0.5 mmol l^{-1}. It is also reduced by non-selective cation current blockers such as 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) and mefenamic acid. In contrast, the Ca^{2+} resting current is potentiated by neurohormone D in a concentration-dependent manner.

The Ca^{2+} resting current seems to play an important physiological role in the control of DUM neurone spike frequency (Wicher et al., 1994) and also in the regulation of intracellular Ca^{2+} concentration *via* a Ca^{2+}-induced Ca^{2+}-released (CICR) mechanism involving ryanodine-sensitive channels rather than inositol trisphosphate channels (Heine and Wicher, 1998).

Na^+ resting current

The Na^+ resting current was described in locust metathoracic ganglion DUM neurones (Goodman and Spitzer, 1981a). This study reported that DUM neurones hyperpolarized by over 10 mV when Na^+ was replaced by Tris or choline, suggesting that the participation of the resting conductance of Na^+ is essential in maintaining the resting membrane potential near −60 mV. Similar observations were made both *in situ* and in isolated cockroach TAG DUM neurones (Lapied et al., 1989). It was shown that the resting membrane potential was dependent on the external Na^+ concentration. The hyperpolarization observed in a reduced external Na^+ concentration was mimicked by external application of saxitoxin or TTX, indicating that most of the resting Na^+ current passed through TTX-sensitive Na^+ channels. Very recently, a detailed analysis of the biophysical properties of this current, using the cell-attached patch-clamp configuration, has allowed a better understanding of its physiological implications for the regulation of DUM

neurone excitability (Lapied et al., 1999). In the range of normal resting membrane potential (−50 mV), the Na^+ current is observed as unclustered single openings. The study of the voltage-dependence of the open probability reveals a bell-shaped voltage-dependence with a maximum open probability at −50 mV. The current–voltage relationship has a slope of 36 pS, and the mean open time constant measured at −50 mV is approximately 0.2 ms. Using a typical scorpion α-toxin, LqhαIT from *Leiurus quinquestriatus hebraeus*, considered to be specific for insect Na^+ channels, it has been possible to obtain further information about the functional characteristics of the resting Na^+ channels. LqhαIT (10 nmol l^{-1}) markedly alters the behaviour of the channels. At −50 mV, the channel activity appears in bursts. This bursting activity is only observed under normal conditions when the membrane is hyperpolarized to levels more negative than −70 mV. The toxin increases the open probability without modifying the voltage-dependence. Interesting results were obtained with higher concentrations of LqhαIT. At 100 nmol l^{-1}, the toxin produces long periods of silence interrupted by bursts of increased channel activity. Interestingly, there is a very good correlation between these bursts and the open probability, which also appears in bursts of high open probability. Whole-cell experiments indicate that application of LqhαIT (100 nmol l^{-1}) to spontaneously active TAG DUM neurones stimulates the transition from rhythmic activity to burst firing (Fig. 2C). This bimodal flexibility is the consequence of an alteration in the behaviour of the resting Na^+ channel (Lapied et al., 1999). These results suggest that the Na^+ resting channels are necessary not only to drive the membrane potential of DUM neurones to threshold for firing spontaneous action potential (Lapied et al., 1989) but are also essential in determining the firing pattern. In other words, these Na^+ channels are essential in

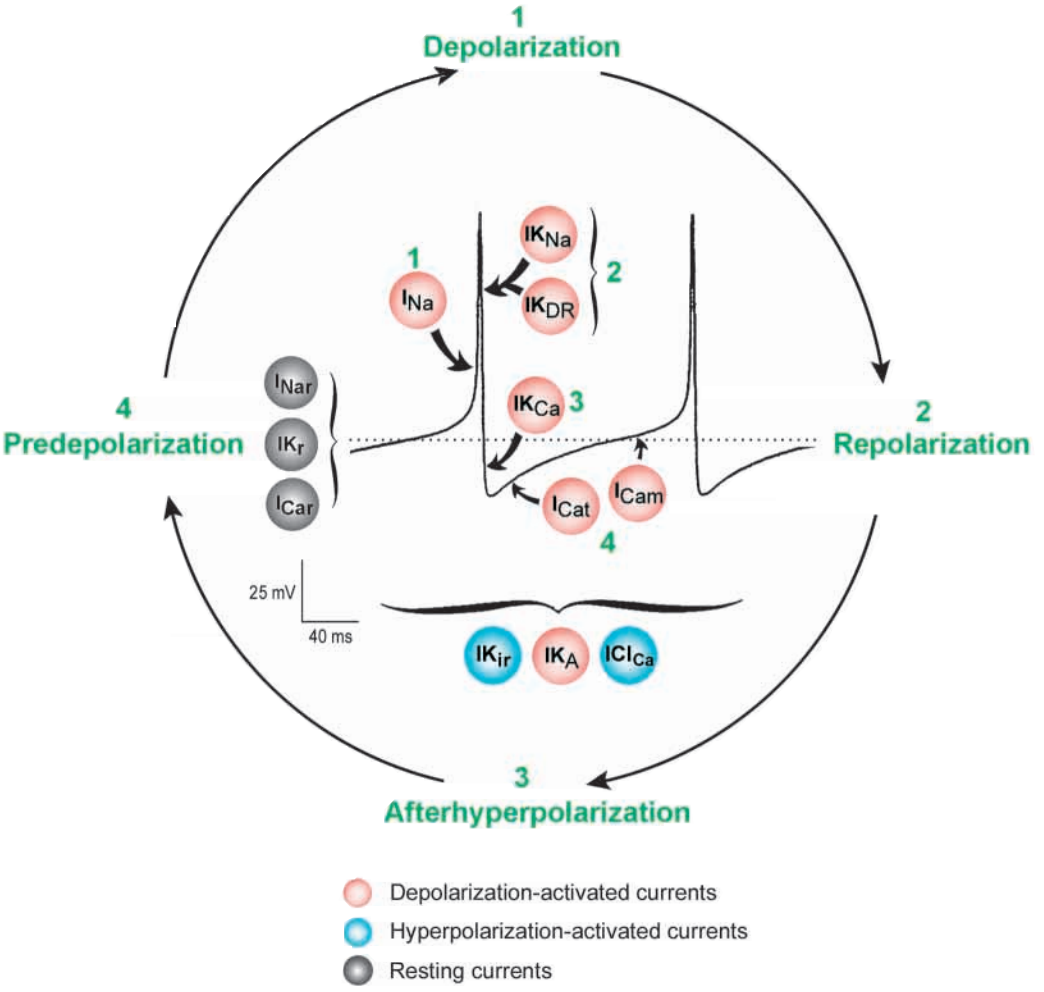

Fig. 3. Intrinsic spontaneous electrical activity in dorsal unpaired median (DUM) neurone cell bodies *in vitro*. A model representing the ionic currents thought to be involved in the generation of the different phases of the beating pacemaker activity. I_{Na} is the voltage-dependent Na^+ current; $I_{K,Na}$, $I_{K,DR}$, $I_{K,Ca}$ and $I_{K,A}$ are the Na^+-dependent K^+ current, the delayed rectifier K^+ current, the Ca^{2+}-activated K^+ currents and the A-like K^+ current, respectively; $I_{Ca,t}$ and $I_{Ca,m}$ are the transient and maintained low-voltage-activated (LVA) Ca^{2+} currents. $I_{K,ir}$ and $I_{Cl,Ca}$ are the hyperpolarization-activated inward K^+ and Ca^{2+}-sensitive Cl^- currents, respectively. These two currents, together with the A-like currents, regulate the firing frequency (horizontal bracket). $I_{Na,r}$, $I_{K,r}$ and $I_{Ca,r}$ are the Na^+, K^+ and Ca^{2+} resting currents. The dotted line represents the putative resting membrane potential, in this model at −50 mV.

maintaining rhythmic pacemaker activity in the normal range of resting membrane potential.

Spontaneous electrical activity from a complex combination of ionic channels

DUM neurones appear to have intrinsic ionic mechanisms capable of producing endogenous oscillatory firing. These mechanisms are triggered at different membrane potential levels and involve a combination of a complex set of ionic currents (Fig. 3). On the basis of the main results presented in this review, it is tempting to propose the following sequence of events underlying spontaneous activity. Note that this is only a hypothetical model restricted to cockroach abdominal ganglion DUM neurones. Additional voltage-clamp and pharmacological investigations, extended to other types of DUM neurone, will provide further information about the complexity of endogenous pacemaker activity.

When DUM neurones are sufficiently depolarized to reach the high threshold of activation of voltage-dependent Na^+ channels (–35 mV), the depolarizing phase of the Na^+-dependent action potential is elicited. Both repolarization and afterhyperpolarization, which follow depolarization, are generated by distinct K^+ channels. The Na^+-dependent K^+ channels, activated in parallel with Na^+ channels, help to limit the duration of the action potential. The delayed rectifier outward K^+ current and particularly the Ca^{2+}-dependent K^+ current contribute to the acceleration of the repolarizing phase and to the generation of the afterhyperpolarization. This afterhyperpolarization is usually deep enough to involve ionic channels that are activated at relatively low potentials. These channels, which are inactive at depolarized membrane potential levels, are de-inactivated when the DUM neurone is hyperpolarized beyond the resting level, the time course being dictated by the closing kinetics of the K^+ channels. The shutdown of the K^+ channels that underlie the repolarization and afterhyperpolarization is sufficient to generate a small depolarization in the activation threshold (–70 mV) of the I_{Ca}tLVA Ca^{2+} current. This Ca^{2+} current, which is involved in the initial part of the predepolarization, is essential in bringing the membrane potential to the threshold of the I_{Ca}mLVA Ca^{2+} current activation (i.e. –60 mV). Finally, this mLVA Ca^{2+} current leads to further depolarization, which allows the activation threshold of the Na^+ channels generating the depolarization of the action potential to be reached (Fig. 3). In parallel, the participation of the resting currents (see above), together with the activation of both A-like K^+- and Ca^{2+}-sensitive Cl^- currents and the inactivation of the I_{Ca}tLVA Ca^{2+} current, will help to regulate the action potential frequency. Furthermore, as we have indicated, Ca^{2+} represents a key factor in the regulation of the spontaneous electrical activity of the DUM neurone. It seems that all Ca^{2+}-sensitive channels (see Table 1) are functional and/or regulated within only a narrow range of intracellular Ca^{2+} concentration. Consequently, Ca^{2+} entering the cytoplasm during DUM neurone activity through resting and voltage-dependent Ca^{2+} channels or following activation of receptor-operated Ca^{2+} channels can act as a direct or indirect (through biochemical pathways) second messenger essential in controlling the firing pattern and, consequently, the neuromodulatory function.

Concluding remarks – future directions

Patch-clamp studies, adapted for dissociated fully differentiated adult DUM neurones, have revealed an unexpected diversity of ionic channels. Most of the channels detected have been characterized with respect to their biophysical and pharmacological properties. The results summarized in this review reveal the complexity of neuronal membrane properties. It can be concluded that DUM neurones have a high degree of specialization in the insect central nervous system. We are only beginning to learn the physiological functions of somatic ionic channels in the generation of spontaneous electrical activity. To help resolve these complex intrinsic membrane properties, mathematical modelling should also provide new insights into the electrophysiological properties of DUM neurones. As previously reported in vertebrate neuronal preparations (Abbott, 1994), these models were able accurately to reproduce or predict new experimental data and/or dynamic neuronal behaviour patterns. However, to be as accurate as possible, such computer models should be based on a wide variety of experimental responses or constraints (e.g. somatic and dendritic anatomy, electrophysiological and molecular data on DUM neurones), many of which should come directly from the extensive literature. These must include data on individual components of the system, such as the kinetics and behaviour of the individual ionic currents, values of conductance and the intracellular concentrations of relevant ions as well as experimental measurements of integrated responses (e.g. action potential frequency, cytoplasmic Ca^{2+} transients).

Although it is well known that spontaneous electrical activity participates in many of the initial events, such as the maturation of neuronal signalling properties or axon outgrowth, in DUM neurones, the physiological role of spontaneous action potentials generated at the somatic level is not well understood. As suggested previously (Hoyle and Dagan, 1978), overshooting action potentials could be required to mobilize a transmitter substance in the soma or to regulate and/or trigger its synthesis. However, we have no direct evidence for this. The use of complementary approaches, including immunocytochemistry and combined voltage- and current-clamp experiments with cytofluorimetry, should help to extend our knowledge in this area. In this way, a fascinating parallel field of investigation could be the study of the ionic mechanisms involved in the generation of spontaneous somatic activity in a developmental context. It is known that both voltage-dependent and voltage-independent ionic channels undergo complex modifications during development. This suggests that the physiological behaviour (i.e. somatic signalling properties) of immature DUM neurones could be different from that of their adult counterparts. It has previously

been demonstrated that some of the phenotypes (e.g. the time course of accumulation of octopamine, changes in nuclear and cytoplasmic volumes, chemosensitivity or axonal outgrowth) expressed by identified DUM neurones change during the course of embryonic development (Goodman, 1982). Consequently, it is reasonable to envisage different patterns of ion channel development in relation to spontaneous somatic activity. It would be interesting to know how embryonic channels might be optimised for their role in mediating spontaneous somatic electrical activity that could influence secretory function and/or axonal outgrowth. In this last case, the development of long-term cultured DUM neurones should provide useful information. Furthermore, another relatively unexplored area concerns the physiological roles of intracellular Ca^{2+} and second-messenger systems in the final developmental events underlying mature electrical activity. As reported previously in other cells (e.g. Desarmenien and Spitzer, 1991; Gu et al., 1994), different second-messenger pathways, such as those involving protein kinases A and C, Ca^{2+}-calmodulin-dependent protein kinases and Ca^{2+}-dependent proteases, seem to play essential roles in the optimisation of the immature ionic channel population. By understanding the functional properties and intracellular regulation of immature ionic channels, which help to mediate the transition between the immature and mature states of excitable cells, we could obtain a better understanding of the physiological function for each of the mature somatic ionic channels mediating the signal-transduction processes involved in secretory function.

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