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SHORT COMMUNICATION

CONOPRESSIN G INCREASES BURSTING ACTIVITY IN COMMAND NEURONS MEDIATING RESPIRATORY PUMPING IN *APLYSIA CALIFORNICA*

BY MANUEL MARTINEZ-PADRON AND KEN LUKOWIAK*

Neuroscience Research Group, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

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Periodic spontaneous gill movements (SGMs) are one of the most obvious of the 21 general action patterns described in the ethogram of *Aplysia californica* (Leonard and Lukowiak, 1986). SGMs are thought to be a prime component of *Aplysia*'s respiratory cycle (Koester *et al.* 1974; Byrne and Koester, 1978) and in the intact animal the frequency of SGMs can be modified by changes in the partial pressures of CO_2 and O_2 of the sea water (Croll, 1985; Levy *et al.* 1989).

SGMs also interact with a number of the avoidance action patterns described in the *Aplysia* ethogram, namely inking (Walters and Erickson, 1986), gill withdrawal and siphon withdrawal (Kanz *et al.* 1979; Pinsker, 1983; Eberly and Pinsker, 1984). In addition, long-lasting changes in SGM frequency occur in response to sudden changes in illumination and food presentation (Eberly *et al.* 1981). Food presentation increases SGM frequency before food contact occurs and only in those animals that subsequently eat (Eberly *et al.* 1981). Finally, increases in SGM frequency may accompany egg laying, since SGM frequency is increased *in vitro* following electrical stimulation of the bag cells (Schaefer and Brownell, 1986). Electrical stimulation of the bag cells leads to egg laying *in vivo* (Ferguson *et al.* 1989). In all these examples, it is not completely clear what mechanism(s) regulates the changes in SGM frequency.

The frequency of SGMs is also increased *in vitro* by the superfusion of conopressin G, a molluscan vasopressin analogue from *Conus geographus* (Cruz *et al.* 1987), over the abdominal ganglion (Martinez-Padron *et al.* 1992*c*). Conopressin G increased SGM frequency up to fourfold and this effect persisted for at least 25min following washout. Recent evidence suggests that a conopressin-like peptide is present in the *Aplysia* nervous system (Martinez-Padron *et al.* 1992*b*; McMaster *et al.* 1992) and may play a role in the mediation of the behavioural state that is associated with food satiation (Martinez-Padron, 1992*a*,*c*).

*Present address and address for reprint requests: Laboratoire de Neurobiologie Cellulaire et Moleculaire, CNRS 91198, Gif-sur-Yvette, France.

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SGMs are triggered by two clusters of small neurons situated at the roots of the branchial and siphon nerves, the R25 and L25 cells (Byrne, 1983; Koester, 1989). These neurons possess endogenous pacemaker activity and make extensive reciprocal electrical and excitatory chemical connections among themselves. Thus, these neurons fire in a highly synchronized population burst that initiates the different components associated with SGM activity.

The present study represents an attempt to analyze the mechanisms by which conopressin G increases SGM frequency. Conopressin G, but not other vertebrate vasopressin-like peptides, increases the burst frequency in the R25/L25 cells. Conopressin G appears to act directly on these neurons and not *via* other neurons (e.g. R20 and R15) that are known to modulate the activity of this network (Alevizos *et al.* 1989, 1991).

Aplysia californica (100–250g, Alacrity) were used in these studies. A more detailed description of the dissection and procedures is given in Martinez-Padron *et al.* (1992*a*,*c*). Briefly, the abdominal ganglion was removed from the anesthetized animal and pinned down on a clear Sylgard (Dow Corning) coated culture dish. To facilitate desheathing, the ganglion was then bathed in artificial sea water (ASW) containing 2% protease type IX (Sigma P-6141) for 1h. Neurons were identified according to their morphology, position and firing properties as described in the literature (Alevizos *et al.* 1989; Byrne, 1983; Koester, 1989). Recordings were made from the R25/L25 respiratory neurons as well as from R20, R15 and bag cells. Single-barrelled micropipettes (15–20 MΩ) filled with 0.8mol l^{-1} KCl were used. The abdominal ganglion was perfused at a constant rate (0.5–3ml min^{-1}) at 18–20°C. Data were stored on floppy diskettes on a digital (Nicolet 2090-III) oscilloscope. In addition, data were recorded with a Vetter digital tape recorder (A. R. Vetter Co.) and played back on a Gould chart recorder. Micromolar concentrations of conopressin G were usually used in these experiments. An effect could be detected at concentrations in the 10 μmol l^{-1} range, but 1 μmol l^{-1} gave the most consistent results. ASW containing conopressin G was continuously superfused over the ganglion for 5min. When TTX was used the perfusion system was turned off.

The effect of bath application of conopressin G (1 μmol l^{-1}) on R25 activity and thus the R25/L25 network is shown in Fig. 1A. Conopressin G caused a marked increase (1.5- to 4.2-fold) in the frequency of R25 bursts. The peptide was also able to elicit bursts of R25 activity in previously quiescent preparations (Fig. 1B). The characteristics of the burst were also affected by the peptide (lower trace), the firing frequency being consistently higher in the presence of conopressin G than in ASW. In all cells tested (N=10) the neurons returned to their control pre-peptide levels of activity within 30–45min following washout.

To determine whether the action of conopressin G is mediated directly onto the R25/L25 neurons, polysynaptic transmission was reduced by bathing the ganglion in either 100 μmol l^{-1} tetrodotoxin (TTX) or high-Ca^{2+}, high-Mg^{2+} ASW (50mmol l^{-1} and 144mmol l^{-1} respectively). TTX completely blocked action potential (AP) generation in R25/L25 neurons and usually revealed the underlying membrane oscillations responsible for the periodic bursting. Under these conditions bath application of conopressin G increased the size, duration and/or frequency of the membrane oscillations in all cells

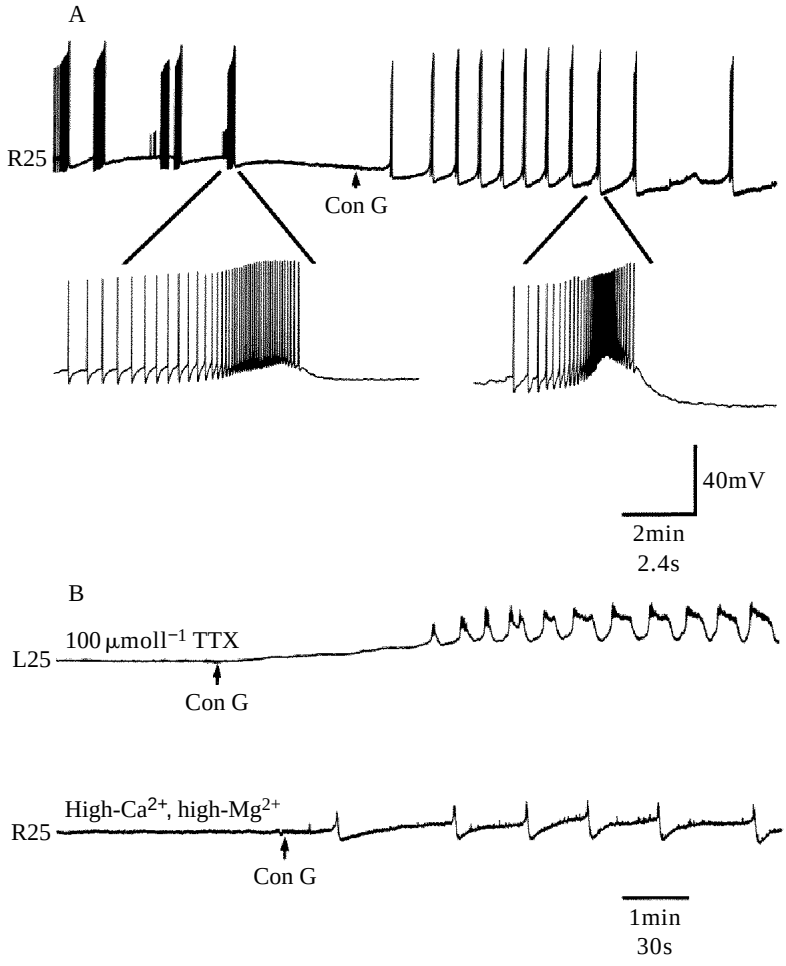

Fig. 1. Representative data from a single experiment showing the effect of conopressin G on spontaneous R25 activity. (A) Bath application of 1 μmoll^{-1} conopressin G increases the frequency of spontaneous bursts approximately twofold (upper trace). In addition, the firing frequency within bursts was consistently higher in conopressin G than in ASW (lower traces). (B) The effect of conopressin G on R25 and L25 appears to be direct. Conopressin G elicited a periodic plateau potential in 100 μmoll^{-1} TTX (upper trace) and weak periodic bursts in high-Ca^{2+}, - high-Mg^{2+} saline (lower trace) in previously quiescent neurons.

tested (*N*=5). In five additional preparations, the cells were quiescent in TTX and conopressin G induced the membrane oscillations (Fig. 1B, upper trace). Spontaneous bursting became infrequent or ceased altogether when the high-Ca^{2+}, high-Mg^{2+} ASW was superfused over the ganglion. Bath application of conopressin G either produced a slow depolarization of the membrane potential (*N*=4 of 4 cells tested) or increased the frequency of spontaneous bursts (*N*=3 of 3 cells tested). As can be seen in Fig. 1B (lower trace), bursts of APs were blocked by high-Ca^{2+}, high-Mg^{2+} ASW and conopressin G triggered a series of weak periodic bursts. Presumably, the high divalent cation ASW prevented the whole R25/L25 network from firing. Thus, a polysynaptic pathway does

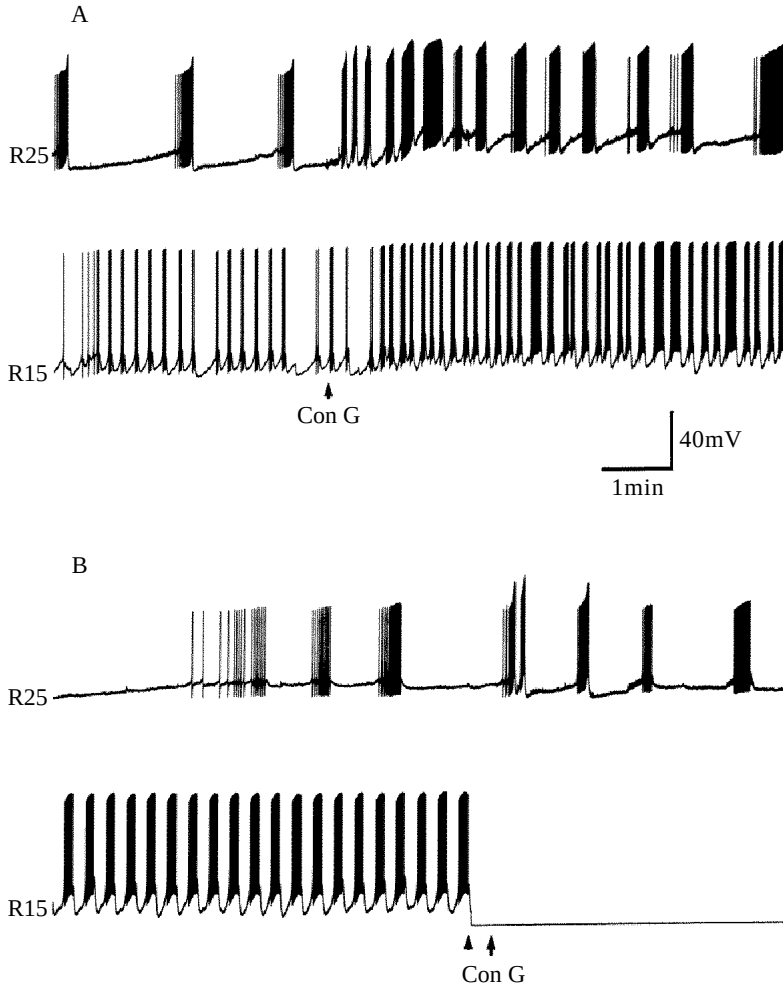

Fig. 2. Excitation of R15 is not required for the effect of conopressin G on R25/L25 cells. (A) Simultaneous recording from R25 and R15. Bath application of 1 μmol l^{-1} conopressin G enhances bursting activity in both cells. (B) In a different preparation where the activity of R25/L25 was lower than that in A, the effect of conopressin G on R25 is still present when R15 is hyperpolarized (arrowhead) before application of the peptide (arrow).

not appear to be necessary for conopressin G to be effective, in marked contrast to some other effects of conopressin G (Martinez-Padron *et al.* 1992*a)*.

Two different types of burst activity are exhibited by the R25/L25 network. When both cell clusters are active simultaneously, strong bursting activity characterized by high-frequency discharges are observed. When only one cluster, usually L25, is fully active the network exhibits more variable and weaker bursts (Koester, 1989). The activity of two types of abdominal ganglion neurons R20 and R15 affects R25/L25 activity in different ways. Induced activity in R20 causes strong bursts in the R25/L25 network (Alevizos *et al.* 1989) while R15 activity causes a longer-lasting increase in the frequency of both

strong and weak bursts (Alevizos *et al.* 1991). We therefore tested whether conopressin G had any affect on these modulatory neurons whilst recording from the R25/L25 network.

Neuron R20 is normally silent and induced activity in it triggers bursts of activity in the R25/L25 network. We used these criteria to identify R20 positively in these experiments. In all three preparations in which R20 and R25 were recorded from simultaneously, superfusion of conopressin G had no effect on R20 membrane potential or synaptic input to it; however, in these three preparations. R25/L25 activity was increased as in Fig. 1A. Further, immunocytochemical experiments do not support the hypothesis that R20 uses a conopressin-like peptide as a transmitter (Martinez-Padron *et al.* 1992*b*). Thus, R20 activity is not necessary for conopressin G's effects to be observed.

Neuron R15 generates endogenous periodic bursts of APs in the isolated abdominal ganglion. Bath application of conopressin G ($1\,\mu mol\,l^{-1}$) increased the frequency of these bursts and the number of APs in each burst (Fig. 2A) in all four neurons tested. Thus, some of the effects of conopressin G on the R25/L25 network might be mediated *via* R15. However, this seemed unlikely since R15's effects on its follower neurons are only apparent when procedures which inhibit transmitter release are utilized (Alevizos *et al.* 1991). Such procedures were not used in our study. In a different preparation from that used in Fig. 2A, conopressin G superfusion continued to increase both the frequency of R25 bursts and the firing frequency within each burst when R15 was hyperpolarized. In the example in Fig. 2B there were two coordinated R25 bursts in the 6min period before perfusion of the peptide and with R15 active, whereas there were five R25 bursts in the first 5min following peptide perfusion when R15 was hyperpolarized. Furthermore, arginine vasotocin (AVT), which also potentiates R15 bursting activity (Moore *et al.* 1981), had no effect on R25/L25 activity.

As mentioned above, bag cell activation increases the frequency of SGMs (Schaefer and Brownell, 1986). This increase in SGM frequency appears to be due to activation of R15 by the bag cells (Alevizos *et al.* 1991). The superfusion of conopressin G, however, had no effect on the bag cells in all four preparations tested, even though R15 burst frequency and/or duration are increased.

Our working hypothesis is that conopressin G or a conopressin-like peptide in the *Aplysia californica* nervous system (Martinez-Padron *et al.* 1992*b*) mediates the effects of food arousal and/or nociceptive stimulation on SGM activity by directly activating the R25/L25 network. In addition, the suppression of other gill withdrawal behaviour patterns associated with food satiation appears to be mediated by the endogenous conopressin-like peptide acting *via* polysynaptic pathways (Martinez-Padron *et al.* 1992*a*).

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