Antagonistic Effects of Phentolamine and Octopamine on Rhythmic Motor Output of Crayfish Thoracic Ganglia

MARK D. GILL AND PETER SKORUPSKI
School of Biological Sciences, Queen Mary and Westfield College, University of London, London E1 4NS, United Kingdom

Gill, Mark D. and Peter Skorupski. Antagonistic effects of phentolamine and octopamine on rhythmic motor output of crayfish thoracic ganglia. J. Neurophysiol. 82: 3586–3589, 1999. Spontaneous rhythmic motor output of crayfish thoracic ganglia consists of bursts of activity in antagonistic leg motor neurons (MNs), alternating with a rather slow cycle period (typically ≥20 s). The most common pattern (77% of preparations) consists of long coxal promotor bursts, the duration of which was correlated strongly with cycle period, and relatively short remotor bursts independent of cycle period. Octopamine, at a concentration of 2–30 \( \mu M \) reversibly retarded this rhythm, increasing both cycle period and promotor burst duration. Higher concentrations of octopamine inhibited promotor nerve activity and abolished rhythmic bursting. Phentolamine (10–50 \( \mu M \)) had the opposite effect of decreasing cycle period, mainly by decreasing promotor burst duration. Whereas in the presence of octopamine promotor bursts were lengthened and became even more strongly related to cycle period, phentolamine promoted a more symmetrical rhythm with shorter promotor bursts that were less dependent on cycle period. When octopamine was applied in the presence of phentolamine, there was no significant increase in cycle period or burst duration, although with shorter promotor bursts that were less dependent on cycle period. Octopamine (an octopamine antagonist in insects) blocks the modulatory effect of octopamine and, interestingly, when applied on its own, modulates spontaneous rhythms in the opposite direction to the effect of octopamine.

INTRODUCTION

The central circuits that generate rhythmic motor output are highly dependent on the prevailing neuromodulatory environment. Experimentally, rhythmic output often is induced by exogenous application of neuromodulators to preparations isolated from their normal inputs. For example, muscarinic agonists induce rhythmic patterns in leg motor neurons (MNs) of crayfish (Chrachri and Clarac 1990) and insect thoracic ganglia (Büschges et al. 1995; Ryckebush and Laurent 1993) and in the crayfish swimmeret system (Braun and Mulloney 1993). In vertebrate spinal cord preparations, excitatory amino acids, aminergic precursors (L-DOPA), serotonin, and dopamine have all been used to induced locomotory rhythms (Cazalets et al. 1992; Kiehn and Kjaerulff 1996). Crayfish isolated thoracic ganglia are also capable of generating rhythmic activity in the absence of exogenous modulators (Sillar and Skorupski 1986; Skorupski 1996), although such a preparation potentially contains endogenous modulators. Spontaneous rhythmic output consists of alternating bursts of impulses in the antagonistic promotor and remotor MNs of the thoracocoxal joint, as during walking in the intact animal, but with a considerably slower cycle period (Chrachri and Clarac 1990; Sillar and Skorupski 1986). In the absence of such activity, the promotor nerve fires continuously and the remotor nerve is silent (Skorupski 1996; Skorupski et al. 1992). The amine octopamine is an inhibitory modulator of rhythmic motor output to crayfish walking legs (Skorupski 1996), as it is also in the segmentally homologous swimmeret system (Mulloney et al. 1987). At high concentrations, octopamine abolishes spontaneous promotor activity (sometimes inducing tonic remotor nerve activity) and rhythmic motor output, if present, ceases. At lower concentrations, octopamine can replace antagonizing promotor and remotor bursts with continuous promotor activity (i.e., convert a rhythmic pattern to a tonic one).

In this paper, we show that octopamine modulates rhythmic cycling in addition to its effects on MN excitability. Phentolamine (an octopamine antagonist in insects) blocks the modulatory effect of octopamine and, interestingly, when applied on its own, modulates spontaneous rhythms in the opposite direction to the effect of octopamine.

METHODS

Experiments were done on adult male and female crayfish, Pacifastacus leniusculus, measuring 8–10 cm rostrum to telson. The preparation was cannulated and perfused with oxygenated crayfish saline, and the thoracic ganglia was dissected out as described previously (Skorupski et al. 1992). The isolated chain of ganglia was then superfused at a flow rate of 1–2 ml/min. Extracellular recordings from cut ends of promotor and remotor nerves of the fourth thoracic ganglion were made using polyethylene suction electrodes. All drugs were purchased from Sigma.

Cycle period (intervals between promotor burst onsets) and promotor burst duration were measured automatically using a Spike2 script (modified from a program by Greg Smith, CED, Cambridge, UK). Burst onset and offset were defined by interspike intervals that (respectively) fell below and exceeded user-defined values (typically, 20–90 ms for burst onset and >180 ms for burst offset). Spikes were discriminated on-line with a 1401-18 window discriminator (CED). Effects on bursting were assessed using a Student’s t-test with the significance level set at \( P = 0.01 \) (unless stated otherwise). Pearson’s linear correlation coefficient was used to evaluate the relationship between cycle period and burst duration. Values in text are given as means ± SE.

RESULTS

Spontaneous rhythmically alternating promotor and remotor bursts occurred with stability sufficient for further analysis in...
Typically, promotor bursts were longer and more variable than remotor. Promotor burst duration was significantly correlated with cycle period in 17 preparations (77%). We will refer to this type of rhythmic bursting activity as type 1. In these preparations, the mean cycle period was 26.64 ± 2.16 s and promotor burst duration was 18.74 ± 2.09 s. Rhythmically active preparations in which promotor burst duration was independent of cycle period are referred to as type 2. The mean cycle period in these preparations did not differ from type 1 (24.24 ± 3.1 s), but promotor burst duration was somewhat shorter (10.81 ± 1.38, P < 0.05, n = 5).

High concentrations of octopamine (100 μM) silence the promotor nerve in both bursting and nonbursting preparations, but lower concentrations can reveal modulatory effects on spontaneous or reflexly generated motor output without abolishing spiking (Gill and Skorupski 1996; Skorupski 1996). We were able to study octopaminergic modulation of rhythmic motor output (as opposed to simply abolishing such activity) in 12 of the 17 preparations that exhibited type 1 bursting, using concentrations of 2–30 μM. In the remaining (type 1) preparations, octopamine either abolished bursting before any other modulatory effect could be revealed (with or without promotor inhibition) or disrupted bursting by inducing erratic activity that could not readily be analyzed.

The principal effect of octopamine on type 1 bursting is an increase in cycle period and promotor burst duration. Figure 1 illustrates a typical experiment, where a 3-min pulse of octopamine (10 μM) leads to a marked slowing of the rhythm. When the octopamine pulse is repeated at 20 μM, the effect is even more marked: the two cycles following octopamine application show approximately fivefold increases in cycle period and promotor burst duration. Figure 1 indicates the effect of octopamine is dose dependent; however, because octopamine concentrations effective for inhibiting bursting (without significantly inhibiting promotor nerve activity) varied from experiment to experiment, we were unable to obtain pooled concentration-response data. Data from all experiments where at least five bursts in octopamine (2–30 μM) could be measured show a significant increase in both cycle period (161.71 ± 15.54%) and promotor burst duration (208.03 ± 29.34%, n = 11). The correlation between cycle period and burst duration also was strengthened by octopamine (Fig. 1, C and D). There was also some shortening of remotor burst duration (85.0 ± 9.54%), although this was less significant (P = 0.046).

The effects of octopamine on type 2 bursting (where promotor burst duration was independent of cycle period) were less consistent. Promotor burst duration was increased in two of these preparations and became significantly correlated with cycle period (P < 0.05; data not shown). Octopamine thus transformed the bursting pattern from type 2 to the more...
common type 1 in these two preparations. However, in three other type 2 preparations, we observed no consistent effects.

Phentolamine, an octopamine antagonist in insects (Evans 1981), only weakly antagonizes the inhibitory effect of octopamine on promotor firing rate (Gill and Skorupski 1996). Interestingly, however, phentolamine (10–50 μM) by itself has an excitatory effect on rhythmic bursting (Fig. 2A), significantly and reversibly reducing both cycle period (63.1 ± 9.2%, n = 8) and promotor burst duration (55.9 ± 10.2%, n = 8). Phentolamine also weakens the relationship between promotor burst duration and cycle period (Fig. 2, B and C).

In four preparations where we tested the effect of octopamine in the presence of phentolamine, the reversible, octopamine-induced increase in cycle period was blocked completely when the same octopamine concentration was reapplied in the presence of phentolamine. This is illustrated in Fig. 3, where the first pulse of octopamine induces a reversible slowing of the rhythmic bursting cycle (Fig. 3A), but a second pulse, 15 min after switching to phentolamine-containing saline, is without effect.

**DISCUSSION**

In preparations generating spontaneous rhythmic activity, the most common pattern is one where relatively long promotor bursts, which are strongly correlated with cycle period, alternate with relatively short remotor bursts, which are independent of cycle period. Octopamine increases the period of this rhythm apparently due to an increase in promotor burst duration. Phentolamine has the reverse effect of decreasing cycle period, mainly or wholly by decreasing promotor burst duration. Octopamine and phentolamine also have opposite effects on the relationship between promotor burst duration and cycle period. Whereas octopamine exaggerates the already marked tendency of the rhythm to be dominated by promotor bursts, phentolamine promotes a more symmetrical rhythm, where the correlation between cycle period and burst duration is weakened (Figs. 1 and 2).

To our knowledge, excitation of a motor rhythm by an amine antagonist (applied alone) has not been reported previously. A possible mechanism is that phentolamine displaces endogenous octopamine from a receptor that mediates octopaminergic inhibition of rhythmic bursting. Alternatively, phentolamine may be acting as an agonist at an unknown receptor type. Phentolamine’s ability to block the effect of exogenous octopamine on spontaneous rhythms (Fig. 3) supports the first possibility. In the same crayfish species, octopaminergic inhibition of swimmeret rhythm is antagonized partially by phentolamine, but here phentolamine by itself tends to disrupt rhythmic activity in contrast to the present results (Mulloney et al. 1987).

In crayfish thoracic ganglia, high concentrations of octopamine (≤ 100 μM) have both excitatory and inhibitory effects on individual remotor MNs and their reflex responses (in correlation with promotor inhibition). The inhibitory, but not the excitatory effects of octopamine are blocked by mianserin (Gill and Skorupski 1996), indicating at least two receptor classes in crayfish CNS. Are the effects on rhythmic bursting mediated by an additional, phentolamine-sensitive octopamine receptor? Alternatively, because crayfish leg MNs have central outputs that may contribute to pattern generation, octopaminergic modulation of the rhythm could be secondary to promotor inhibition. This now seems unlikely because the modulation can occur without promotor inhibition (Fig. 1, A and B). Furthermore, mianserin by itself has no rhythm-enhancing effect (Gill and Skorupski 1996).

Phentolamine and mianserin are insect octopamine antagonists (of somewhat variable potency and selectivity) at class 2 and 3 receptors (Evans 1981; Roeder 1994). If phentolamine exerts its rhythm-enhancing effect in crayfish by acting as an octopamine antagonist, the question arises as to the source of endogenous octopamine. In lobster, octopamine-containing cells are present in brain, suboesophageal and thoracic ganglia (Schneider et al. 1992). In the preparation used here, segmentally repeated thoracic clusters of these cells should be preserved. In view of the present results, it would be very interesting to determine if these cells are spontaneously active as is the case with lobster serotonergic cells (Ma et al. 1992).

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Address reprint requests to P. Skorupski.

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