

## INFLUENCE OF OXYGEN ON THE HEARTBEAT RHYTHM OF THE LEECH

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### SUMMARY

1. Raising and lowering the oxygen content in the fluid bathing the skin of the leech modified the frequency of the heartbeat. In raised concentrations of oxygen, the period between bursts of impulses in the heart excitor motoneurons (HE cells) was reduced. Conversely, with lowered oxygen concentrations bathing the skin, the heart rate was slowed, with longer periods between bursts of firing in the HE motoneurons.

2. Changes in oxygen concentration did not affect HE motoneurone firing patterns in preparations in which the CNS was dissected from the skin and surrounding body tissues.

3. Various other stimuli were tried, including stroking, pinching, stretching the skin, blood vessels and gut, as well as changing the temperature. None of these stimuli mimicked the specific effects of oxygen on rhythmicity.

4. It is concluded that peripheral receptors sensitive to changes in oxygen tension are able to influence the central neuronal circuits responsible for generating the rhythm of the heartbeat.

### INTRODUCTION

In a series of studies, the rhythmical contractions of the heartbeat of the leech have been explained in terms of the activity of a well-defined group of nerve cells within the CNS (Thompson & Stent, 1976*b,c*; Calabrese & Peterson, 1983). Three different types of neurones have been identified by their morphology, locations and electrophysiological properties (Thompson & Stent, 1976*a,b*; Calabrese & Maranto, 1984). These are the heart motoneurons (HE cells), the heart interneurons (HN cells) and the heart accessory cells (HA cells), the latter exerting a modulatory role. The rhythm is established in the HE motoneurons, which are not rhythmically active on their own, by periodic bursts of inhibitory postsynaptic potentials (IPSPs) from the HN interneurons. Thus, the interconnections of the HN cells are responsible for generating the pattern of the heartbeat (Peterson & Calabrese, 1982).

Key words: leech, heartbeat, oxygen receptors.

Arbas & Calabrese (1984) have shown that certain conditions can influence the heart rate. For example, changes in the temperature of the fluid bathing the CNS, activation of swimming, mechanical stimulation of the skin and impulses in single sensory cells influence the rhythmical contractions of the heart tubes. The aim of the present experiments was to determine whether another physiological variable, oxygen tension, could influence rhythmicity of the muscle contractions. The results show that changes in oxygen tension in the fluid bathing the skin have a marked effect on the rhythm. This effect is graded, occurs in response to changes in oxygen at physiological levels, and appears to be mediated by peripheral receptors acting on the central oscillators.

#### MATERIALS AND METHODS

Conventional intracellular recordings were made from HE motoneurons using microelectrodes filled with  $4 \text{ mol l}^{-1}$  potassium acetate. The preparation consisted of a chain of four or five ganglia still connected to the intact head end of the leech (*Hirudo medicinalis*), innervated by the head ganglion and the first seven segmental ganglia. The fluids bathing the leech head were separated from those bathing the ganglia by a Vaseline-sealed, plastic partition. This made it possible for chemical stimuli to be applied to the skin without affecting the ganglia in which recordings were made (see Fig. 1). Oxygenation of the fluid was varied by bubbling different concentrations of oxygen or nitrogen through Ringer solution contained in a reservoir and delivered to the preparation at a constant rate. Measurements of the pH and partial pressures of oxygen and carbon dioxide showed that the fluid delivered to the front end of the preparation did not affect the fluid in the recording side of the chamber. The partial pressure of oxygen (measured by the Stanford University Medical Center Blood Gas Laboratory) ranged from values of 623.5 to 21.0 Torr with a constant pH of 7.4, corrected to 25°C, when 100% oxygen and 100% nitrogen, respectively, were bubbled into the reservoir. When these values were compared to atmospheric oxygen tension measured in Ringer solution, the addition of oxygen represented an increase of 481.4 Torr, whereas the reduction in oxygen represented a decrease of 121.1 Torr.

In other experiments, a second preparation was used. This was a single ganglion (in this case either ganglion 8, 9, 10 or 11), still attached on one side to a piece of body wall with all three major peripheral nerve roots (Nicholls & Baylor, 1968).

#### RESULTS

When the oxygen content of the fluid bathing the skin was changed, a difference in the firing pattern of the HE motoneurons was observed. Fig. 1 shows the increase in cycling frequency produced by raised oxygen. At the same time, there was an increased amplitude of the depolarizing to hyperpolarizing excursions. The effects of raised oxygen were graded and depended on the concentration applied to areas of skin distant from the ganglion (Fig. 2). Decreasing the oxygen concentration in the bathing fluid by bubbling nitrogen into the perfusion reservoir slowed the cycling

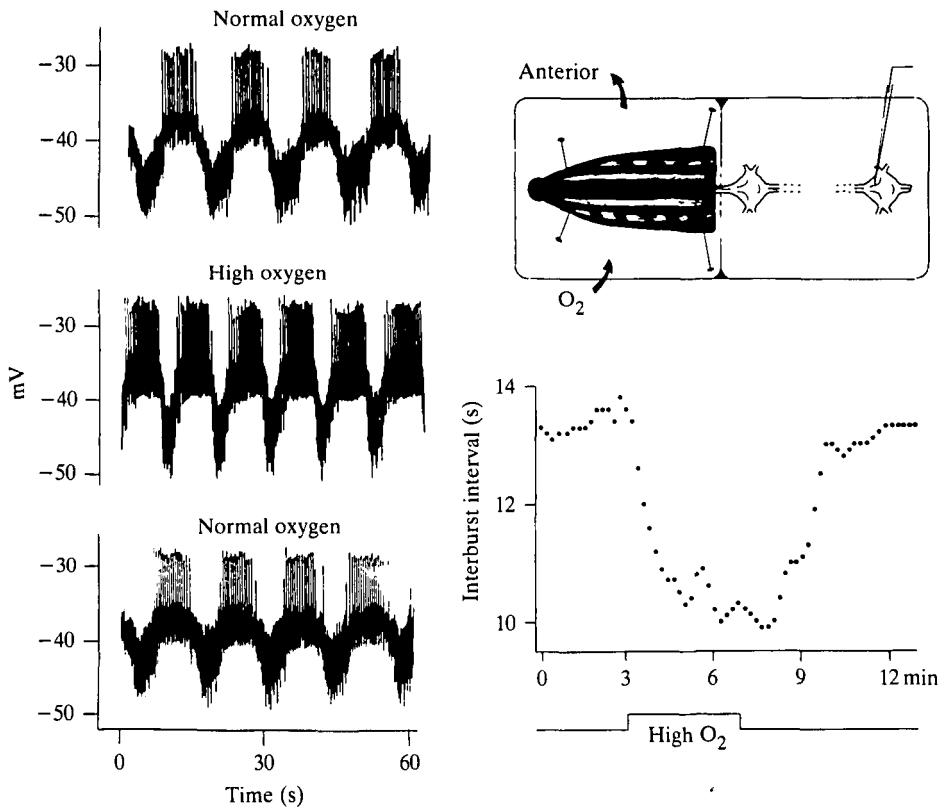


Fig. 1. Intracellular recordings, experimental preparation and cycle period plotted as a function of oxygen concentration. Intracellular measurements were taken from an HE motoneurone situated in a mid-body ganglion (segmental ganglion no. 12). The experimental preparation (not drawn to scale) represents a leech head, approximately 3 cm in length, bathed in fluid which was separated from that bathing the attached chain of segmental ganglia by a Vaseline-sealed, plastic partition. The arrows indicate the side of the chamber in which fluids were perfused.

frequency (Fig. 3) and reduced the amplitude of the depolarizing to hyperpolarizing excursions.

These effects were not seen when ganglia at the front end of the animal were directly exposed to changes in oxygen concentration, without the surrounding tissue and body wall (Fig. 2). In such preparations, consisting of CNS on its own, the cycling frequency at rest increased by about 50 % when compared to preparations with skin attached. Although changes in oxygen produced no effect in such preparations, faster rates could still be induced by manipulations such as cutting connectives (showing that the cycling rates were not maximal). Similarly, nitrogen bubbled into the fluid reservoirs did not slow the cycling frequency unless skin was present.

A variety of different stimuli were tested for their effectiveness in altering the cycling of HE motoneurons. None produced effects similar to those following changes in oxygen tension. In preparations that had only a single ganglion attached to

the body wall, pinching, pressure and stretching the skin, dorsal and lateral heart tubes and gut caused no consistent changes in rhythmicity. Stimulating the receptive field of a single touch-sensitive sensory neurone (T cell) evoked an increase in HE motoneurone cycling frequency at the end of the stimulus. This effect is consistent with the results of Arbas & Calabrese (1984), who showed that activation of individual T cells by touch or by direct electrical stimulation increased the cycling frequency of the HE motoneurone. However, when the skin of an intact head-end preparation was stroked with long sweeping movements, activating many sensory receptors sequentially, a different response was observed: HE motoneurons became hyperpolarized, while the cycling frequency remained constant (Fig. 4).

Changing the temperature in the fluid bathing the leech head also showed clear effects on rhythmicity. As expected (Arbas & Calabrese, 1984), heating the fluid to 35°C caused an increasing frequency of cycling while cooling the fluid to 5°C caused a slowing. However, these responses, unlike those seen with changes in oxygen tension, were not necessarily mediated by temperature receptors in the skin or gut, because when a chain of ganglia was exposed directly to altered temperatures, similar responses were observed.

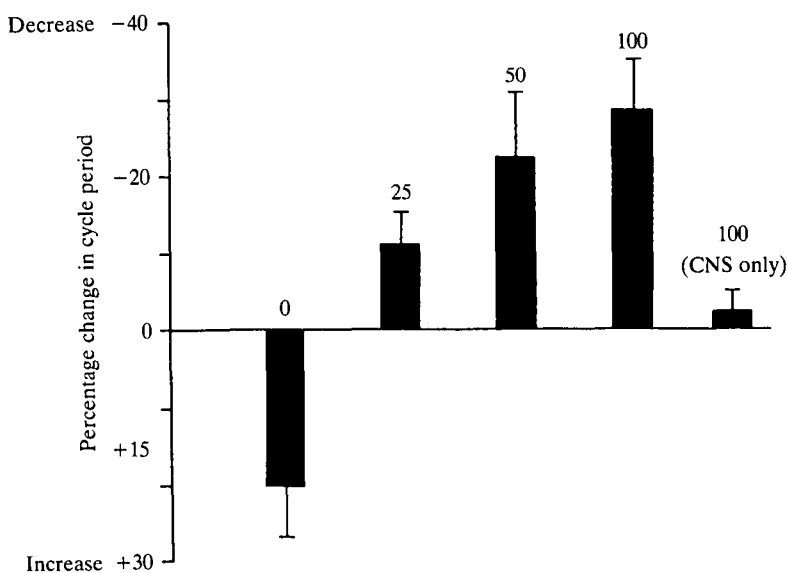


Fig. 2. The percentage of oxygen bubbled into a reservoir containing Ringer solution which was perfused over the head end of the leech is shown on the abscissa (0% oxygen was achieved by bubbling 100% nitrogen into the reservoir). In all cases, except the bar on the extreme right (which illustrates the results from isolated CNS), the intact head-end preparation, illustrated in Fig. 1, was used. The percentage change in the cycle period from baseline (Ringer solution at atmospheric oxygen tension) to the cycling period maintained during exposure to each of the four test solutions is expressed on the ordinate. The results for 0%, 25%, 50% and 100% (CNS only) oxygen groups were extracted from a total of 16 experiments, four for each category. Nine experiments composed the 100% category using the intact head preparation. The standard error of the mean is depicted as an error bar.

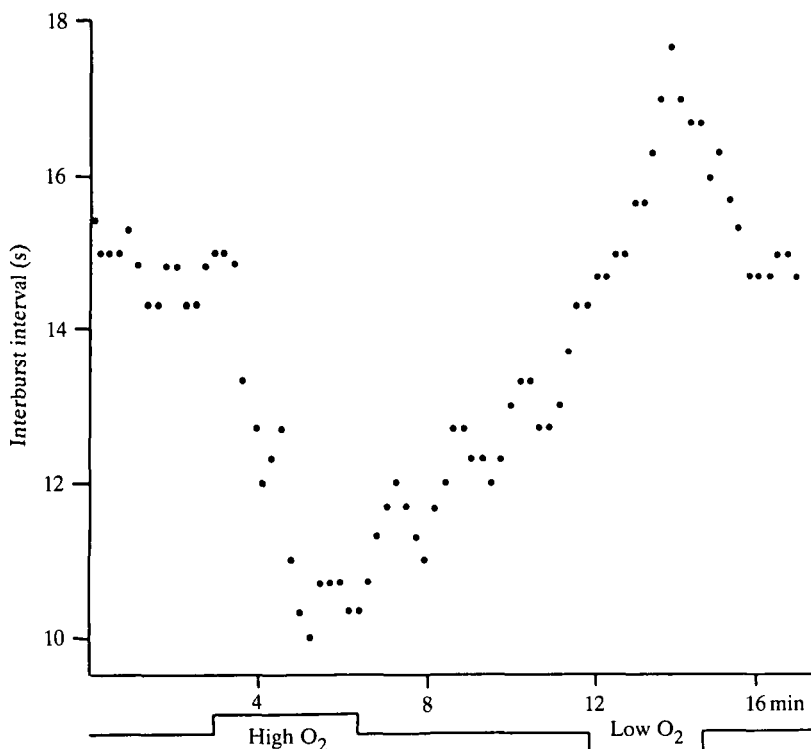


Fig. 3. Cycle period plotted as a function of oxygen concentration from one experiment in which the preparation was exposed first to an increase and then a decrease in oxygen tension. The conditions of maximum and minimum oxygen tension were approximated by bubbling either 100% oxygen or 100% nitrogen into the Ringer solution.

#### DISCUSSION

Neurones within the CNS that can generate the rhythm essential for the heartbeat of the leech can do so in the absence of sensory stimulation or peripheral reflexes (Thompson & Stent, 1976a). However, as in other systems that have been studied in which rhythm is generated centrally (for reviews see Friesen & Stent, 1978; Cohen, 1979; Delcomyn, 1980; Kristan, 1980; Grillner, 1985; Selverston & Moulins, 1985), sensory inputs can influence the character of the rhythm, its period and its strength (Wyse & Page, 1976; Anderson, Forssberg, Grillner & Wallen, 1981; Sigvardt & Mulloney, 1982; Pearson, Reye & Robertson, 1983; Wendler, 1983; Arbas & Calabrese, 1984; Lennard, 1985). For the experimental preparation used in the present experiments, Arbas & Calabrese showed that a whole array of stimuli (many of which activated the neural programme for swimming) could produce a change in the rhythm of the HE motoneurone. For example, tactile stimuli applied to the leech skin, or even activation of a single T cell electrically, produced an increase in the frequency of the rhythm. In the present experiments, mechanical stimuli also influenced the firing pattern of the HE motoneurones; however, in the intact head-end preparation, the extensive sensory stimuli involving large areas of skin produced instead a hyperpolarization. This response may have been mediated either by

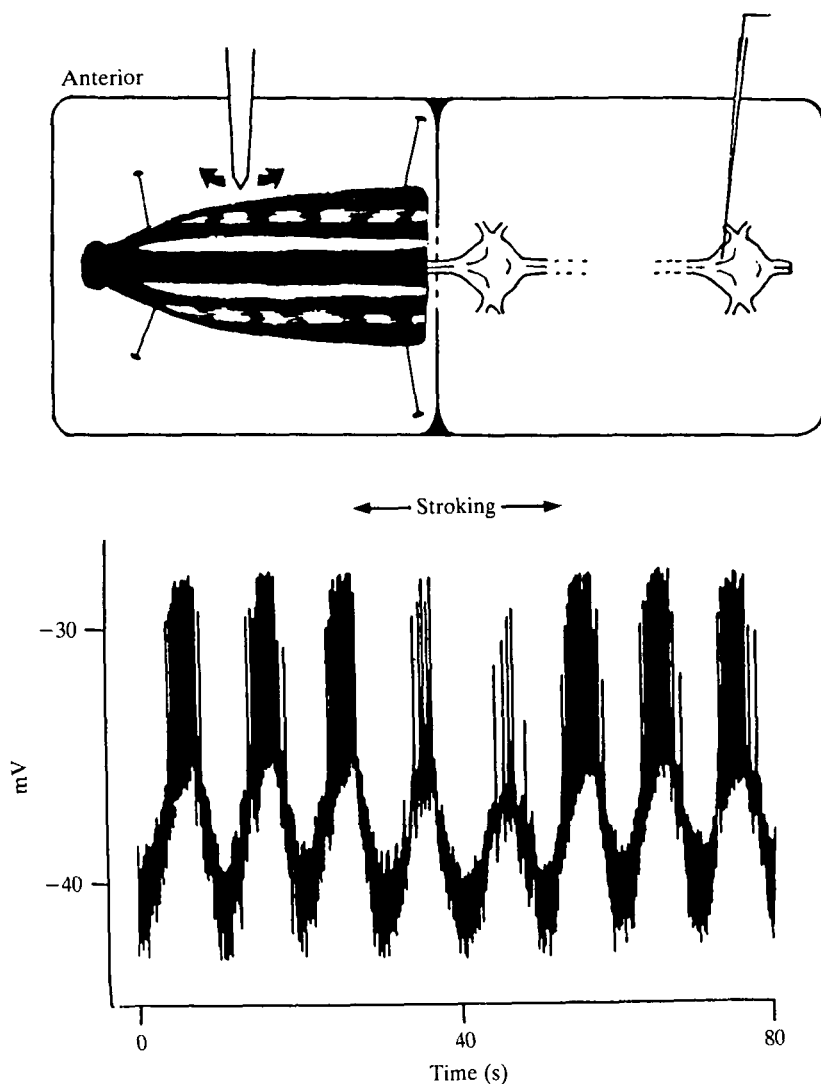


Fig. 4. Experimental preparation and an intracellular record from a mechanical stimulation experiment. The record was taken from an HE motoneurone situated in a mid-body ganglion (segmental ganglion no. 10).

different receptors (e.g. the water movement-sensitive sensilla described by Phillips & Friesen, 1982) or by activation of large numbers of T cells in adjacent segments.

For a leech in its natural environment, salinity and temperature, as well as the processes of photosynthesis and respiration of aquatic organisms, could all affect oxygen (and carbon dioxide) concentrations in the animal's fluid environment (Dejours, 1979). What is clear from the present experiments is that oxygen tension changes the synaptically imposed rhythm of the HE motoneurons and therefore the heart muscle contractions. This stimulus did not directly affect the neurones within the CNS and is thought to be mediated by peripheral receptors. Thus, the characteristic changes in frequency (which showed that the interneurons producing

the rhythm had been influenced) were only produced if the animal's skin was left attached to the CNS. In addition, the increased cyclic frequency observed after removing the skin and surrounding tissues is further evidence of the modulatory role of the periphery.

At present there is no evidence about the nature of these receptors or their locations. It is tempting to conclude that in an intact animal, these inputs from the postulated oxygen receptors would allow the heart rate to be regulated in response to changes in the fluid environment.

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