

CHARACTERISTICS OF MECHANORECEPTORS IN THE AIR-BREATHING ORGAN OF THE HOLOSTEAN FISH, *AMIA CALVA*

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SUMMARY

Single nerve fibre discharge was recorded from mechanoreceptors associated with the air-breathing organ in double-pithed specimens of the bowfin, *Amia calva* L. These receptors were innervated by the vagus nerve and although their exact location was difficult to determine, most appeared to be located along the antero-ventral wall of the single lung. All receptors increased tonic discharge with step increases in lung volume, above a threshold level, and were slowly adapting. There was a dynamic, rate-sensitive burst of activity associated with lung inflation and a dynamic, rate-sensitive inhibition of discharge associated with deflation. These responses were qualitatively similar to those of the tonic stretch receptors found in fish swimbladder and mammalian gut. All receptors were insensitive to changes in intrapulmonary partial pressures of oxygen and carbon dioxide. These observations suggest that receptors capable of transducing the rate, as well as the degree, of inflation and deflation are associated with primitive lungs, and may have arisen from tonic gut receptors.

INTRODUCTION

Air breathing has evolved many times in the lower vertebrates with a variety of structural adaptations giving rise to gas exchange organs. Those found among the air-breathing fishes are extremely diverse. Most species of air-breathing fishes rely on a variety of modifications of the buccal cavity and pharynx, on the walls of the stomach and intestine, on air sacs or on lungs for aerial exchange. The mechanisms used to ventilate these gas exchange organs are equally diverse as, undoubtedly, are the control mechanisms involved (see Gans, 1970 for review).

Various cardiovascular and respiratory reflexes have been described which are associated with inflation and/or deflation of these organs (see Johansen, 1970, 1972; Wood & Lenfant, 1976 for reviews; Johansen, Hanson & Lenfant, 1970; Babiker, 1979; Smatresk & Cameron, 1982; Pack, Galante & Fishman, 1984). Furthermore, changes in volume of gas exchange organs have been implicated in adjusting breathing frequency to match metabolic rate (Johansen, 1970; Smatresk & Cameron, 1982) and buoyancy state (Gee & Graham, 1978; Gee, 1981). Such correlations between

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changes in lung volume, metabolic rate, buoyancy state, breathing frequency and cardiac output resulting from both lung inflation and deflation led Johansen (1970) to speculate that, if these vagus-mediated reflexes are homologous with the Hering-Breuer reflex found in mammals, the situation found in air-breathing fishes may exemplify a basic reflex that exerted a more important regulatory role early in its phylogenetic history.

Such observations and speculation suggest the presence of receptors in the gas exchange organs of fishes which convey volume-related information *via* vagal afferent fibres. Recently, both slowly adapting and rapidly adapting mechanoreceptors were described in the lungs of the dipnoan lungfish *Protopterus* and *Leipidosiren* (DeLaney *et al.* 1983), confirming these predictions. These receptors possessed discharge characteristics similar to those described for frogs (Taglietti & Casella, 1966; McKean, 1969; Milsom & Jones, 1977) suggesting phylogenetic continuity of receptor types and transduction properties from lunged fishes to terrestrial vertebrates. Given the diverse and independent origins of air-breathing organs in different groups of fish, the question then arises: how uniform are the discharge characteristics found in receptors associated with these organs? Are pulmonary receptors of the type described in the dipnoan lungfish and amphibians a natural correlate of the evolution of sac-like gas-exchange organs or are they peculiar, among fishes, to the sarcopterygian line which gave rise to the terrestrial vertebrates? The present study was designed to answer these questions by examining the air-breathing organ of the primitive holostean fish *Amia calva*, a descendant of the actinopterygian line of fishes which gave rise to the teleosts, for the presence of lung receptors, and, if such receptors were present, to characterize their discharge patterns.

METHODS

Observations were made on 10 bowfin, *Amia calva*, obtained from Lake Erie by commercial fishermen (Port Colburn, Ontario) and air-freighted to the University of British Columbia. They were held outdoors in large fibreglass tanks supplied with running, dechlorinated water (5–8 °C) and kept on a natural photoperiod. Several days to 1 week before experiments were run, the fish were moved to indoor tanks and maintained at room temperature (20–23 °C) and a 12 h light : 12 h dark photoperiod. Room temperature acclimation was found to be essential for successful recording.

Surgical procedures and recording techniques

All experiments were performed on double pithed animals which were placed on their backs on a holding platform and covered with cotton soaked in saline to keep them moist. A small opening was made in the left-hand body wall on the ventral surface just behind the cleithrum exposing the anterior portion of the body cavity. The oesophagus was opened by a midventral incision revealing the dorsal, glottal opening to the single lung. The lung was then intubated with a polyvinyl catheter that fitted snugly through the glottal sphincter and was held in place by a purse string suture. A side arm of this catheter allowed tracheal pressure measurement (Statham P23Db pressure transducer and Gould transducer amplifier) and gas sampling (Beckman OM-11 and LB-2 gas analysers). Because the body cavity was open to the

atmosphere throughout these experiments, intrapulmonary pressure was equal to transpulmonary pressure.

On leaving the cranial cavity, the vagus nerve on each side provides branches to the ipsilateral gill arches as it courses along the top of the gill arches below the floor of the cardinal sinus. As it emerges into the body cavity, the vagus follows the intestinal vein down the anterior medial wall to the dorso-lateral side of the oesophagus. This is the ramus intestinalis of the vagus which divides providing innervation to the lung and viscera (Fig. 1A). In the present studies, the ramus intestinalis was isolated on the left side where it crosses from the posterior cardinal sinus to join the oesophagus (Fig. 1A). The nerve was dissected free of surrounding tissue, placed on a dissecting platform and desheathed. Small filaments were dissected from the distal end of a small cut made in the nerve and single unit action potentials from afferent fibres were recorded by conventional means using bipolar platinum electrodes. This activity was amplified using a Framp (F. M. Smith, Vancouver, B. C., Canada) preamplifier (PRA-2) and amplifier (GPA-2), whose frequency bandwidth was set from 20 Hz to 10 KHz. The amplifier output was further filtered with a 60 Hz notch filter. Both the unfiltered preamplifier output and filtered amplifier output were monitored with an oscilloscope and audio-amplifier. The filtered signal was also connected to a window discriminator (Frederick Haer & Co.) and instantaneous rate meter (EKEG Electronics, Vancouver, B. C., Canada). The filtered and unfiltered signals and the window discriminator output were stored on magnetic tape (Tandberg Series 115 Instrumentation Tape Recorder) along with the intrapulmonary pressure signal. The output of the window discriminator, instantaneous rate meter and pressure transducer were also recorded on a Beckman Type R Dynograph recorder.

Location of pulmonary receptors

Receptor discharge was attributed to pulmonary stretch receptors if the discharge was modulated by artificial ventilation. No rapidly adapting receptors were observed in these experiments; all receptors were either slow or non-adapting during maintained lung inflation. For further confirmation, following each experimental run, the lateral body wall of each fish was cut open, the lung exposed and the response of each unit to punctate stimulation of the lung determined (Fig. 1C). All fibres which fired in synchrony with lung inflation responded to discrete local stimulation of the lung or glottal walls with a fine probe and were thus assumed to arise from pulmonary receptors.

Experimental protocol

While monitoring the discharge of each receptor, the bowfin were ventilated with a positive pressure respirator (Harvard Inc.) using mixtures of humidified air containing 0, 5 or 10 % CO₂, or 10 % CO₂ in N₂ at pump frequencies from 5 to 20 min⁻¹ and tidal volumes of 10–25 ml. The pump was stopped to allow the lung to equilibrate to atmospheric pressure or hold inflation constant at various volumes. The lungs were also inflated and deflated in a step-wise fashion using a syringe in place of the pump. The effect of changes in the rate of step inflation or deflation was also determined using a syringe.

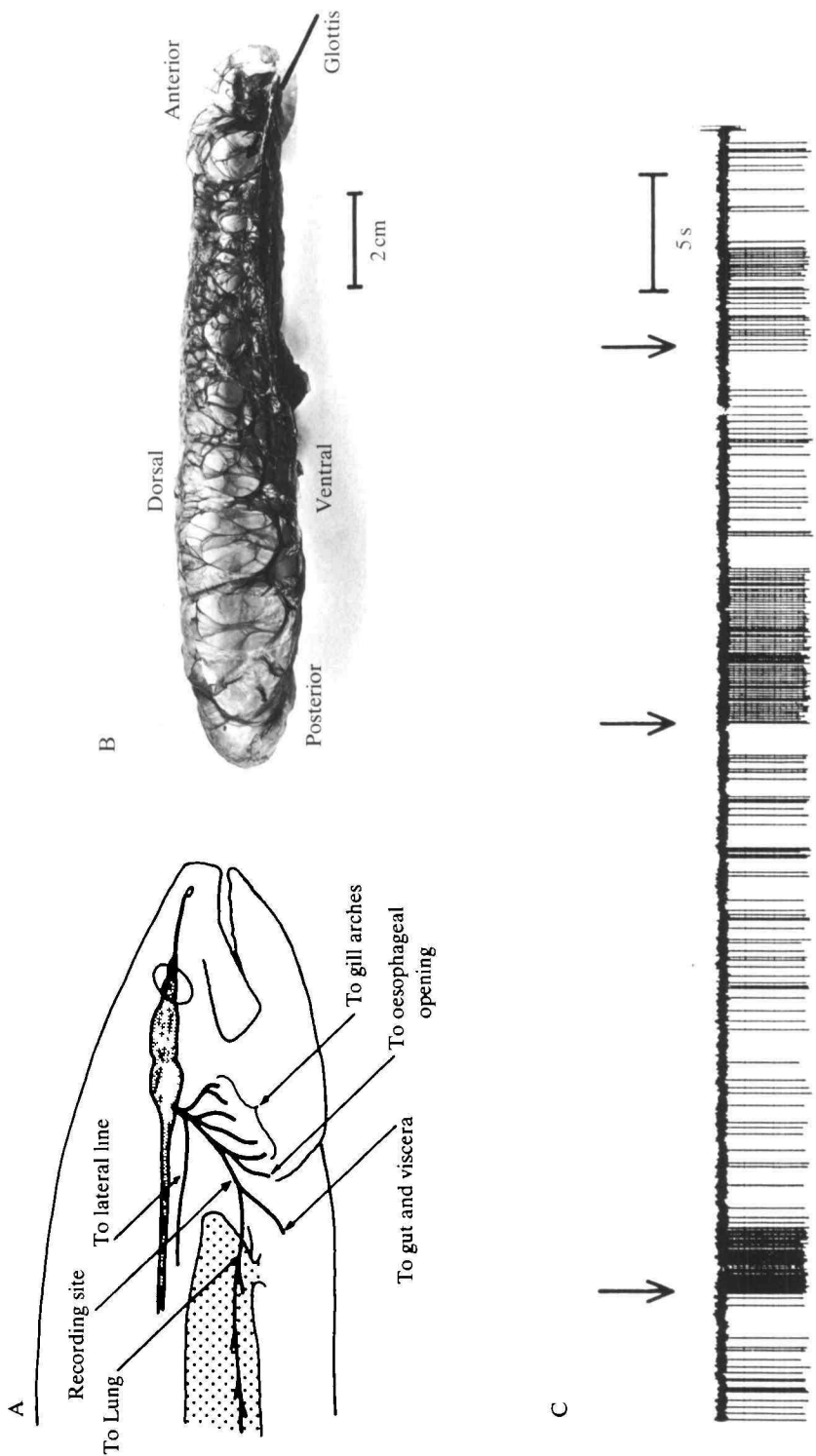


Fig. 1. (A) Schematic diagram illustrating the course of the vagus nerve and the area from which recordings were made. (B) An air-dried lung of *Ambia* in cross section illustrating internal septation and region of glottal opening. (C) Discharge of a pulmonary stretch receptor in response to punctate stimulation (arrows) of the lung.

RESULTS

A total of 10 single-fibre preparations were obtained in this study from receptors located in the lung (Fig. 1B). Their exact location, however, was difficult to determine. One receptor was positively located in the glottal lips and another was located in the general area of the dorsal lung wall. The others were associated with the ventral wall of the lung along its anterior portion where it lies juxtaposed to the gut to which it is joined by a connective tissue sheet. It was too difficult to dissect the lung free of the gut in this region without disturbing the preparation and therefore impossible to determine whether the receptors lay in the outer lung wall or along one of the internal septa (Fig. 1).

The volume threshold for discharge in these units was quite variable: several were active when the lungs were open to atmospheric pressure, others exhibited activity only after the lungs had been inflated to varying degrees (5–25 ml). Consequently, the average discharge frequency at any given volume was also highly variable from fibre to fibre (25 to 300 impulses min^{-1} at 35 ml inflation volume, for instance). All fibres

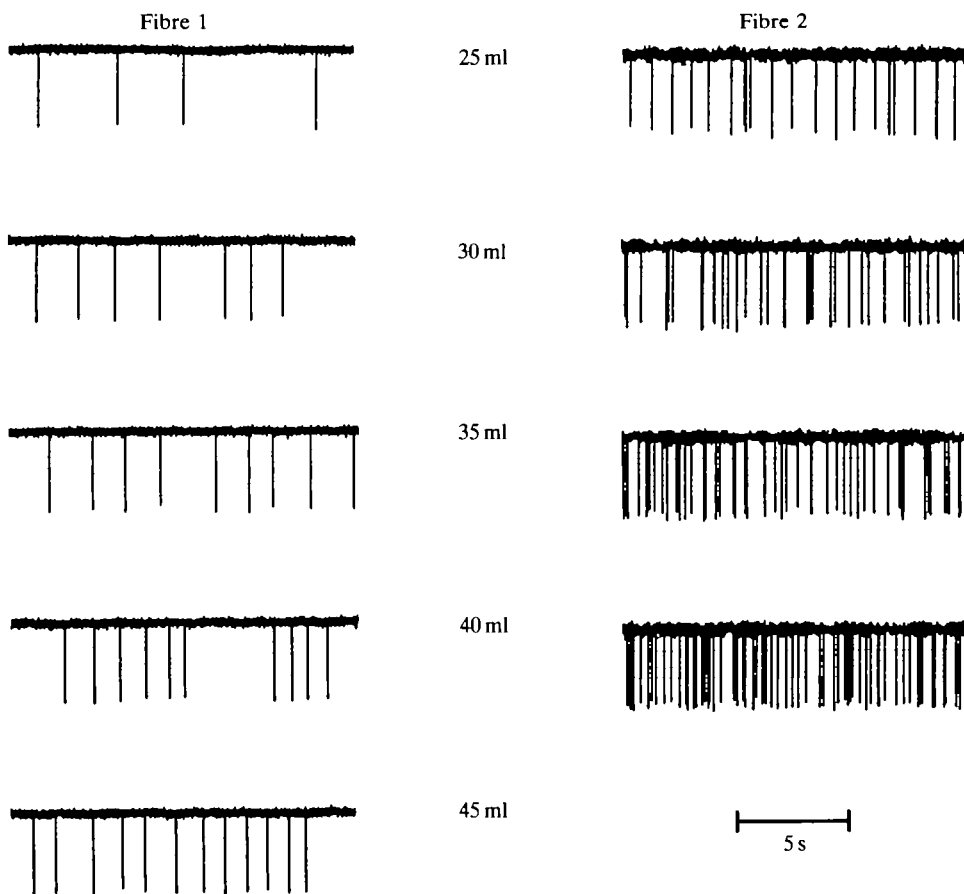


Fig. 2. The effect of maintained lung inflation at various lung volumes on the discharge of two slowly adapting pulmonary receptors.

exhibited some adaptation to a step increase in volume over the range from 5 to 60 ml, but the rate of adaptation estimated from Index 1 of Davis, Fowler & Lambert (1956) was always less than 25 %. After adaptation had occurred, the steady discharge of these fibres in response to maintained lung inflation at all volumes above threshold volume (or end-expiratory volume in those fibres active when the lung was open to the atmosphere) increased with increasing volume (Fig. 2). Neither the relationship between receptor discharge and lung volume nor that between discharge and pulmonary

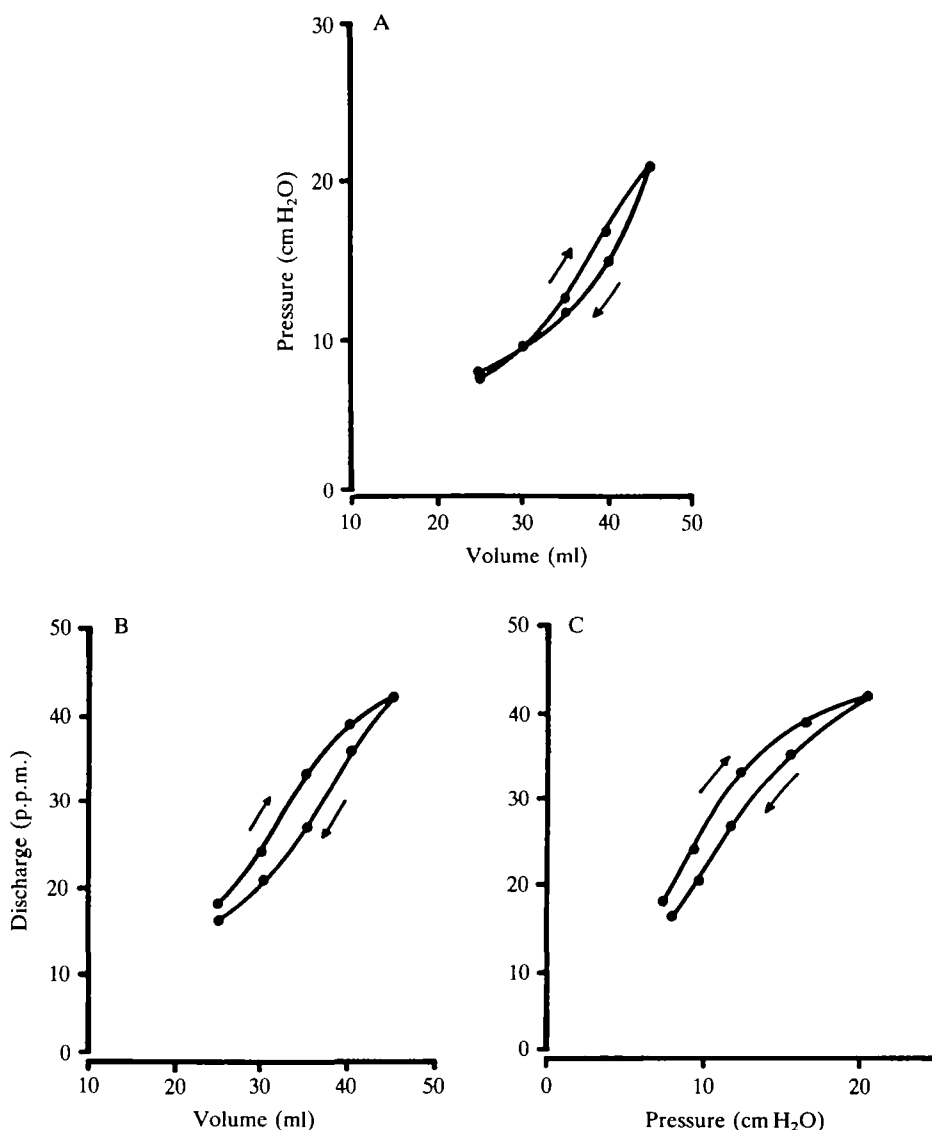


Fig. 3. The relationships between changes in transpulmonary pressure, inflation volume and pulmonary stretch receptor discharge during step inflation and deflation of the lung. (A) Plot of pulmonary pressure *versus* inflation volume. (B) Plot of discharge frequency of a single stretch receptor *versus* lung volume during the same inflation-deflation sequence. p.p.m., pulse per minute. (C) Plot of discharge frequency *versus* pulmonary pressure from the same receptor and during the same inflation-deflation sequence as in A and B. The time relationship of each plot is indicated by the arrows.

pressure was linear, however. In two fibres, the levels of discharge associated with each lung volume during step deflation of the lung from maximum inflation were compared with the discharge levels obtained during step inflation of the lung to the same volumes. At any given volume, discharge was generally less during step deflation (Fig. 3B). A hysteresis of roughly the same magnitude and direction was also present when discharge was plotted against pulmonary pressure (Fig. 3C). Surprisingly, there was little hysteresis in the pressure-volume relationship (Fig. 3A).

During lung inflation, maximum discharge in all fibres was a function of both the rate and volume of inflation giving rise to a rate-dependent overshoot in activity or 'on-response' (Fig. 4). Despite this, discharge in any given fibre fell to the same level within approximately 5 s of the start of a maintained inflation regardless of the inflation rate. End-expiratory discharge rate in these fibres was also a function of the rate and volume of deflation (Fig. 4). This dynamic, rate-sensitive inhibition of discharge (undershoot or 'off-response') also decayed over time with discharge at any given volume returning to the same levels regardless of the rate of deflation (Fig. 4).

The dynamic responses of these units to pump ventilation were similar to those for

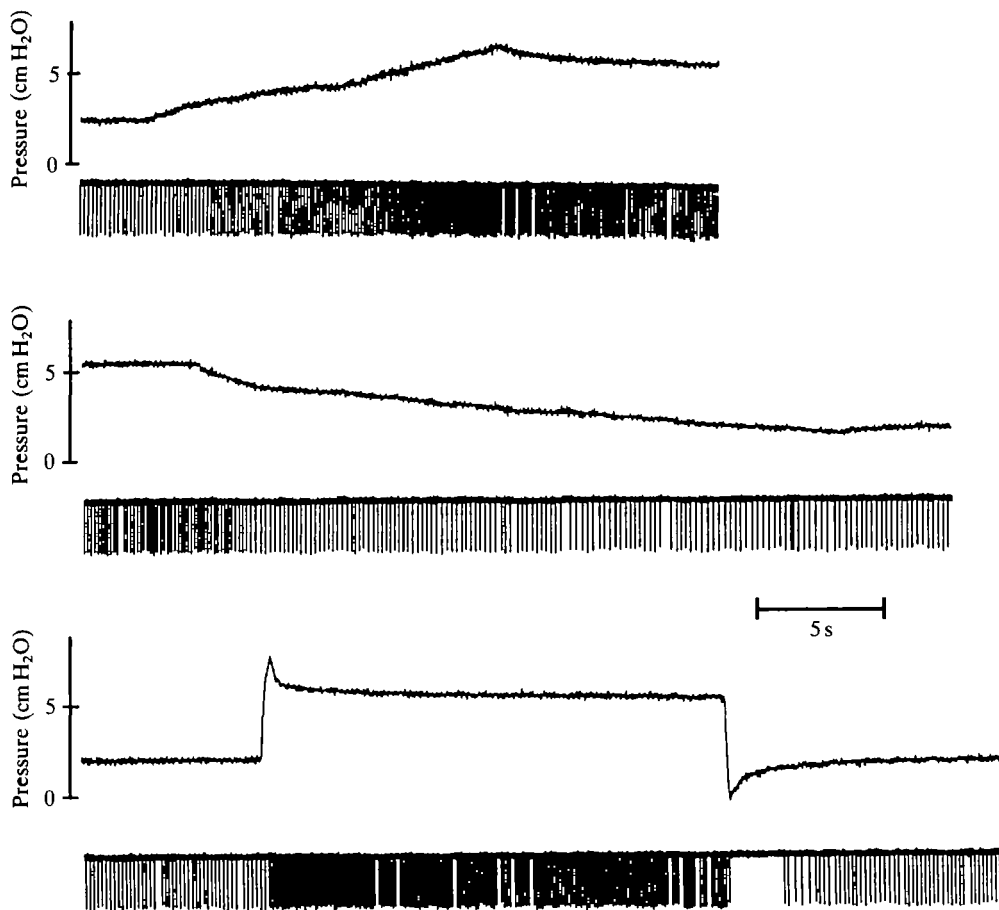


Fig. 4. The effect of changing the rate of lung inflation and deflation on pulmonary pressure and the discharge rate of a single pulmonary stretch receptor.



Fig. 5. The effect of pump ventilation of the lungs with tidal volumes of 15 and 25 ml on pulmonary pressure and the discharge rate of a single pulmonary stretch receptor.

static inflation and deflation. There was an increase in discharge associated with an increase in the volume and rate of inflation (Figs 5, 6). The discharge associated with the first pump ventilation following a prolonged period of maintained deflation, however, was always greater than that associated with subsequent pump ventilations of a continuing sequence at the same volume. Because of the 'off-response' associated with higher rates of deflation, either increases in tidal volume and therefore the rate of passive deflation due to elastic recoil (Fig. 5), or ventilation rate, would decrease end-expiratory discharge. This could eliminate all tonic activity during the expiratory phase of the ventilation cycle in those fibres which were still active when the lungs were open to atmosphere (Fig. 5).

Neither increasing levels of inspired CO₂ nor reduced levels of inspired O₂ had any effect on receptor discharge during pump ventilation over periods of 5 to 10 min (Fig. 6).

DISCUSSION

The results of this study demonstrate the presence, in *Amia*, of slowly adapting mechanoreceptors whose discharge is modulated by static and dynamic changes in lung volume and with afferent fibres running in the vagus nerve.

Although we were not able to determine the exact nature of the stimulus modality of these receptors with our experimental protocol, indirect evidence suggests that it may be lung wall tension as has been suggested for pulmonary stretch receptors of frogs (Taglietti & Casella, 1966; McKean, 1969). In *Amia*, the relationships between receptor discharge and lung volume and pulmonary pressure both showed hysteresis during step deflation of the lung when compared with step inflation. At any given volume or pressure, discharge was always less when the lungs were deflated to that condition from a larger volume or higher pressure than when they were inflated to that condition from a smaller volume or lower pressure. In the dipnoan lungfish *Protopterus* and *Lepidosiren*, Delaney *et al.* (1983) also found a hysteresis in the relationship between lung volume and the discharge of slowly adapting pulmonary receptors. Although these authors conclude that transpulmonary pressure is the adequate

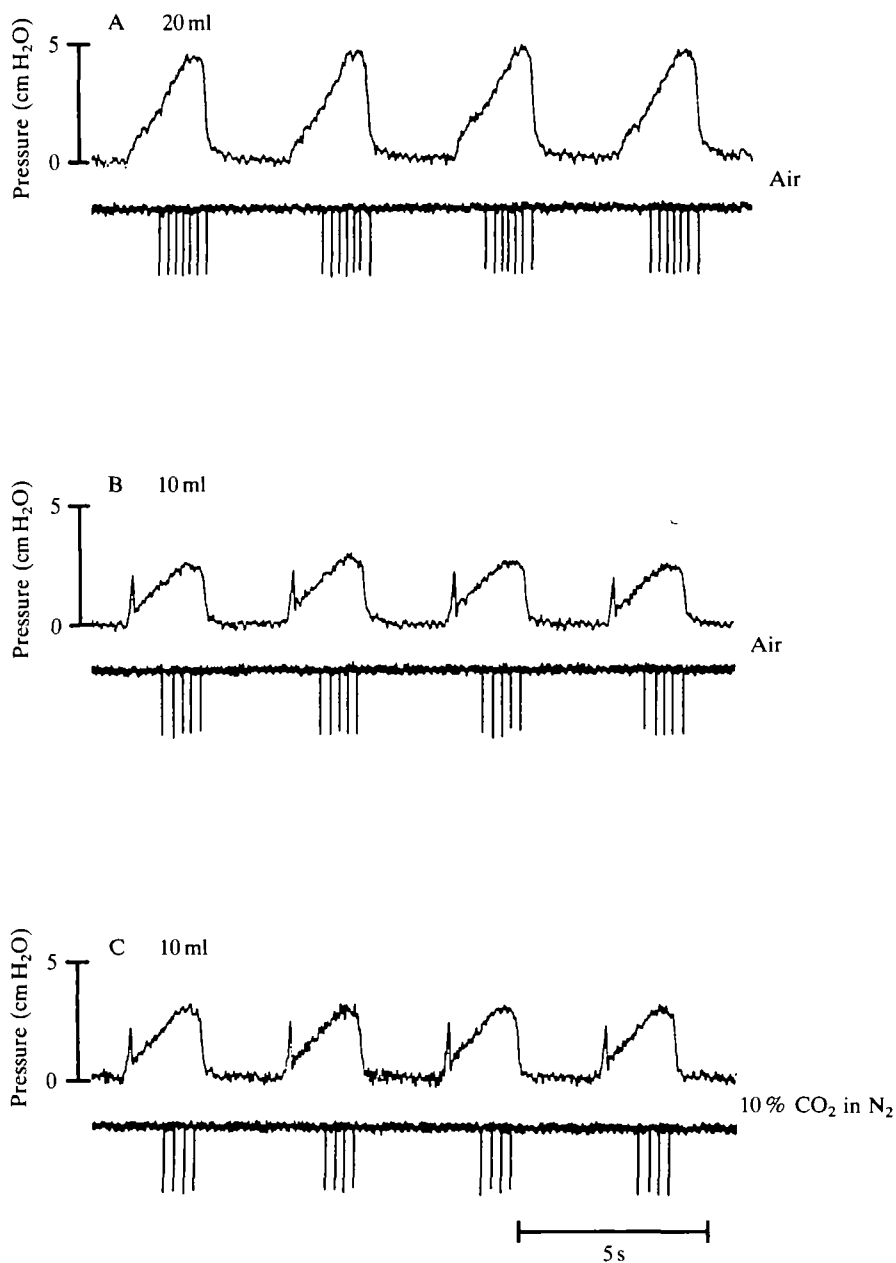


Fig. 6. The effect of pump ventilation of the lungs with different tidal volumes (A,B) and different gas mixtures at the same tidal volume (B,C) on pulmonary pressure and the discharge rate of a single pulmonary stretch receptor.

stimulus for these receptors, replotting the data in their Fig. 8 reveals there is also a hysteresis in the relationship between transpulmonary pressure and receptor discharge. These data suggest that neither the lung volume nor the transpulmonary

pressure can be the adequate stimulus for the slowly adapting pulmonary stretch receptors in *Amia* or the dipnoan lungfish. It remains possible, therefore, that lung wall tension, which is a function of lung volume, pulmonary pressure and lung surface area (Taglietti & Casella, 1966; McKean, 1969), is the stimulus modality for pulmonary receptors in the holosteans and dipnoans as it is in the amphibia.

A major difference between the characteristics of the receptors in the *Amia* lung and those in other lower vertebrate lungs is the apparent lack of any inhibitory effect of CO₂ on the discharge of receptors in the *Amia* lung. Although the effect of CO₂ on discharge of pulmonary receptors in lungfish, amphibia and reptiles was highly variable from fibre to fibre (see Jones & Milsom, 1982 for review) and the apparent lack of effect in *Amia* could simply stem from the small sample size rather than from any real functional difference, we do not feel this is the case. The consistent absence of any effect in all fibres examined is strong evidence that CO₂ does not have an inhibitory effect on these receptors. Another difference between the pulmonary stretch receptors of *Amia* and those of other vertebrates would appear to be the rate-sensitive inhibition of discharge associated with lung deflation. This is a characteristic these receptors share in common with slowly adapting receptors in the swim bladder of the cyprinid fishes *Leuciscus* and *Scardinius* (Qutob, 1962) as well as with most vertebrate tonic receptors such as those associated with photoreceptors, carotid sinus baroreceptors and muscle and gut stretch receptors (Patton, 1965). Given the origins of the swimbladder and lung as outpocketings of the gut (Romer, 1970), this is not too surprising. The off-effect seen in tonic receptor discharge has a correlate in a hyperpolarizing off-effect in the stretch receptor generator potential. To what extent this hyperpolarization reflects changes in the capacitance properties of the receptor ending itself or changes in non-neural elements around the receptor ending, is unclear (Patton, 1965). Thus, whether the absence of such a response in the pulmonary stretch receptors of other vertebrates is a true difference in the receptors themselves or simply a difference in the mechanical linkage between the receptors and the lung or tracheal walls remains an interesting question.

During pump ventilation of the *Amia* lung, the dynamic (phasic) characteristics of receptor discharge were predominant in establishing overall levels of receptor activity. Peak discharge rates during lung inflation were strongly influenced by the rate of inflation and end-expiratory discharge rates were more a function of deflation rate than lung volume. In spontaneously breathing *Amia*, however, lung ventilation is usually a solitary event beginning with an active expiration followed by inspiration and breath holding (Johansen *et al.* 1970). In this context, if these receptors are functionally equivalent to the pulmonary stretch receptors of higher vertebrates, it is quite possible that their dynamic discharge characteristics play an important role in establishing the timing of the events associated with ventilation. It is more likely, however, that the cardio-respiratory and buoyancy-related reflexes associated with changes in lung volume during the period of breath holding (Johansen, 1970, 1972; Johansen *et al.* 1970; Wood & Lenfant, 1976) are a consequence of changes in the tonic level of receptor discharge, although such speculation must remain highly conjectural until a more direct link is established between these receptors and any functional events.

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