RESPIRATORY REFLEXES IN THE DOGFISH

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The classical experiments of Hering & Breuer (1868) and of Head (1889) demonstrated that inflation of the lungs in mammals resulted in inhibition of inspiration, and that this response was abolished by vagotomy. The Hering-Breuer reflex subsequently came to be regarded as of crucial importance in the generation of respiratory rhythms. The vagal inhibitory inflow, augmented by that from a supra-medullary brain-stem centre, was believed to segment the output of a tonically active inspiratory centre into a series of separate respiratory acts (Pitts, Magoun & Ranson, 1939). Although it is now generally believed that the mammalian medulla deprived of both vagal and supra-medullary influences is capable of initiating cyclic breathing, the vagal inflow is still thought to be important in regulating the pattern of respiration (Breckenridge & Hoff, 1950). The possibility that afferent activity initiated by inspiration and carried by the vagus might be concerned in the regulation of branchial respiration in fish has not previously been explored. In this paper the existence of such reflex regulation is reported from experiments on twenty-five dogfish. A brief report of this work has already appeared (Satchell, 1958).

Specimens of the dogfish _Squalus lebruni_ (Vaillant) were fixed by a system of rigidly clamped brass pins which screwed into the cartilage in front of the eye and above the auditory capsule. The trunk was similarly fixed at six places along its length. The fish was perfused through the mouth with sea water, cooled sufficiently to maintain the body temperature between 8 and 12° C.

In some preliminary experiments the outputs of two sensitive strain gauges, connected to the spiracle closer and the pharyngeal wall respectively, were fed into separate d.c.-coupled amplifiers and displayed on a double-beam oscilloscope. Since the movement of the pharynx always followed the movement of the spiracle, both in timing and in amplitude, the movement of the spiracle alone was recorded in subsequent experiments and the lower beam was used to monitor inflation. This was effected by an air-filled balloon in the pharynx connected both to a hand bulb, and, by a side arm, to a tambour pressing against the strain gauge. Very brief periods of inflation were produced by an electromagnetic device either manually controlled or triggered from the sweep. Nerve discharges were recorded with external platinum leads and displayed after a.c.-coupled amplification; they were also monitored with a loud-speaker. In one experiment records of single respiratory neuron discharges were made with Ling-Gerard glass micropipettes. Records were taken with a Grass camera (model C.4.D.) on continuously moving film.
The response to pharyngeal inflation

A pharyngeal inflation of moderate duration (19–20 sec.) reduced both the rate and amplitude of respirations during the period of inflation. Fig. 1 A–C shows three successive inflations of increasing extent. The response to a small inflation was a reduction in the rate of respiration, but the amplitude was little altered. With larger inflations the individual respirations became increasingly shallow. After deflation, and occasionally after inflation, the fish made one or more expulsive movements of the pharynx in which water was shot out, not only from the gills but also from the mouth and spiracle. This expulsive reflex has been commented upon by several previous workers (references in Brown, 1957). It occurs spontaneously in aquarium fish, and can be evoked by stimulation of any part of the body. It is thought that the reflex serves to rid the gills of foreign matter. This reflex was recorded in the experiments as a deep respiration (Fig. 1 B, C); it induced a further period of inhibition which was succeeded by a period of hyperpnoea with respirations of increased amplitude. Although inflations were frequently terminated by this expulsive effort, it was not always present.

![Fig. 1. A–C, the response to successively greater inflations; D, the response to inflation, from another experiment. Top beam: spiracle closer movement; bottom beam: inflation. Time, sec.](image)

A feature noticeable in Fig. 1 A–C, but demonstrated better in Fig. 1 D from a different experiment, is that the inhibition was more obvious at the start and finish of an inflation than it was during the period of inflation. The significance of this will be discussed when the discharges in the vagal afferent fibres are considered.

That the reduction in amplitude of respiration during inflation was not due to faulty recording resulting from skeletal distortion caused by the inflated balloon was demonstrated in two ways. By means of Ling-Gerard type glass micropipettes records of the discharges of respiratory motor neurons were made. It was possible to locate the motor nucleus of the VIIth nerve as some of the cells penetrated could be fired antidromically (i.e. with a latency of 0.5–1 msec.) by stimulating the hyo-
mandibular nerve. Inflations resulted in a reduction in the bursts of discharge of these VIIth nerve motoneurons from 5 to 7 at each inspiration to 2 or 1 or none at all. After deflation they started firing again as before. In other experiments, recordings of the discharge in the branchial branches of the vagus were made from a fine twig dissected free and mounted on recording leads. Here again, during inflation, the bursts of discharges were reduced in duration.

The response to tetanic stimulation of a branchial branch of the vagus

A tetanic stimulus (square waves, 7 msec. duration, 15/sec.) applied to the central end of any of the cut branchial nerves caused a more or less complete inhibition of respiration depending on the strength. Fig. 2D, E shows the response to tetanic stimulation of the left 3rd branchial branch of vagus. With a weak stimulus slow respiration started up again after 16 sec.; with a stronger stimulus (Fig. 2E) inhibition was complete and some effect persisted for two or three respirations after the end of the stimulus. As with inflation, a brief period of hyperpnoea supervened after the inhibition.

Fig. 2. A–C, the response to tetanic stimulation of the cornea of the eye, with successively stronger shocks; D, E, stimulation of the left 3rd branchial branch of vagus. Top beam; spiracle closer movement; bottom beam; duration of tetanic stimulation. Time, sec.

Is the inhibition following inflation due to some non-specific stimulation?

It is well known that in mammals the stimulation of pain fibres may cause either inhibition or acceleration of respiration. In the experiments it was found that any nocuous stimulus to the fish, such as stroking the cornea, pricking, or tetanizing an afferent nerve, also had both inhibitory and acceleratory effects on respiration. In Fig. 2A–C are seen the responses to the tetanic stimulation of the cornea of the left side, with successively stronger shocks. With a weak shock there was a brief period of inhibition at the start of the stimulus. In Fig. 2B this period of inhibition was succeeded by three inspirations that are slightly more rapid than before. In Fig. 2C with a still stronger shock there was a disorganization of respiration with breaths of different size and inhibitory events mixed up with excitatory ones. A comparison of this trace with that in 2 D or E, where a branch of the vagus was being stimulated, makes it clear that the inhibition that results from branchial nerve stimulation or
inflation of the pharynx is to be distinguished from that arising from stimulation of other receptive fields. It was never possible to produce the complete and sustained inhibition mediated by the vagus from nerves anatomically unrelated to the respiratory apparatus.

Discharges in vagal afferent fibres

If inflation produces inhibition by firing pharyngeal receptors which send their afferent fibres along the branchial nerves, then it should be possible to record the discharges of these fibres in the peripheral end of a cut branchial nerve. In such a preparation any activity recorded must originate peripherally and be destined for transmission to the medulla. Fig. 3A, B shows records obtained from a fine twig split off the main trunk of the peripheral cut end of the left 3rd branchial branch of
the vagus. The responses to two inflations of different extent are illustrated. The water was turned off for the few seconds whilst the record was taken as there are receptors in the pharynx which fire continuously when the water is flowing, and they obscure the response to inflation. That inflation causes receptors to fire is clear; two sorts of discharge can be recognized. There was a firing when the balloon was being inflated and deflated, and a less vigorous sustained firing during the period of inflation. It is probable that one arises from receptors stimulated by movement, and the other more specifically from tension. If the pattern of discharges in Fig. 3A is compared with the pattern of inhibition in Fig. 1D a resemblance is seen. The bursts of activity at the moments of inflation and deflation correspond with the more intense inhibition at these times, whilst the sustained activity between could account for the lesser degree of inhibition during the period of inflation.

That these receptors are fired by the normal respiration occurring during an experiment is demonstrated in Fig. 3C, where the lower beam was used to monitor the spiracular closer. There was a small cluster of action potentials at the apex of each inspiration; their firing also caused rhythmic bursts of sounds on the audio channel, each synchronous with pharyngeal movement. As can be seen in Fig. 4, where both spiracular closure and pharyngeal contraction were recorded simultaneously, the closure of the spiracle preceded the pharyngeal contraction by approximately 0.1 sec. Hence the discharges of the receptors recorded in Fig. 3C, were really occurring during the active phase of pharyngeal contraction rather than at its peak.

The dependence of the inhibitory effect on the time of onset of a brief inflation

By using an electromagnetic inflating device it was possible to inflate the balloon for a period as short as 200 msec. and to 'place' this inflation at any chosen position in the respiratory cycle. From a series of more than 100 such inflations it became clear that they were equally effective in any position during the inter-respiratory pause, but were ineffective or much less effective if they occurred during the rising phase of an inspiration (Fig. 4A, B). This is interpreted as indicating that the firing of receptors or the occlusion of their afferent pathways at inspiration makes them less able to respond to an artificial inflation at this time. When the pharynx is once more at rest inflation results in additional inhibitory impulses passing up the branchial nerves and so delays the onset of the next inspiration.

The influence of the activity relayed in branchial afferents on the pattern of respiration

No differences in the manner and tempo of respiration were detectable by direct observation of fish under aquarium conditions and under experimental conditions. In normal respiration each respiratory movement is followed by a pause (Fig. 5A). When the anterior cardinal sinus of each side was opened and all the branchial branches of the IXth and Xth nerves cut the pattern promptly changed to that seen in Fig. 5B. Inspiration followed expiration without intervening pause and the rate of respiration increased. That this change was not simply due to circulatory in-
sufficiency resulting from opening the sinuses was shown in a sham experiment. Both sinuses were opened and the superficial ophthalmic nerves were cut. All the branchial nerves were left intact. Even 3 hr. later the respiratory pattern had not changed, although the rate had slowed slightly. As circulatory failure sets in, the respiration tends to slow down rather than to accelerate, but the inter-respiratory pause remains.

Fig. 4. The response to a brief inflation. Top beam; movement of spiracle closer. Bottom beam; movement of pharynx. The black line indicates the position and duration of the inflation in the respiratory cycle. Time, 1/10 sec.

A second method of demonstrating the change in respiration that results when the respiratory centres are deprived of the inhibitory inflow from the branchial nerves was to paralyse the respiratory muscles by a slow injection of tubocurarine, whilst simultaneously recording the contraction of the spiracle closer and the motor discharges in a dissected twig of the vagus. As the curare took effect and the respiratory movements declined in amplitude, the respiratory rate increased. The strain gauge recorded sixteen successively smaller respirations between the onset of curarization and complete paralysis and during this time the respiratory rate increased steadily from 34/min. to 44/min. The stream of sea water supplied the fish with oxygen, and records were still being taken three hours later so the acceleration was unlikely to have been due to anoxia. Curare may exert some specific effect on the cells of the respiratory centre, but the similarity between these results and those of branchial nerve section suggests rather that curare freed the respiratory centres from the inhibitory discharges engendered by respiratory movement.

That the acceleration following section of the IXth and Xth nerves was due to the elimination of most of the inhibitory feed-back was further shown by stimulation of the proximal end of a cut branchial branch. In Fig. 6A and B the restoration of
the inter-respiratory pause by a repeated single stimulus to the proximal end of the 3rd left branchial branch of vagus is shown. It was possible to evoke pauses of varying length depending on the strength of the stimulus to the nerve: it was not found possible to restore the pauses by continuous tetanic stimulation of the vagus, although many combinations of strength and frequency were tried.

![Graph showing changing respiratory pattern and rate](image)

Fig. 5. The change in respiratory pattern and rate resulting from section of the branchial branches of IXth and Xth nerves. A, before section; B, after section. Time, 10 sec.

The influence of the pre-medullary centres on the respiratory pattern

In mammals the existence of a pontine facilitatory centre has long been known (Lumsden, 1923). In the dogfish transection between the medulla and the mesencephalon (the pons is vestigial in elasmobranch fish) leads to no marked change in normal respiration. The rate was sometimes a little slower, but the most noticeable result was an increased sensitivity to the effects of pharyngeal inflation. In some fish a moderate inflation would inhibit inspiration completely, a result never seen in fish with an intact brain. There is thus some evidence that centres rostral to the medulla exert an influence antagonistic to vagally relayed inhibition. Brain transection combined with vagotomy resulted in the same pattern of rapid respiration with no pause between breaths such as was seen with vagotomy alone. There was never any development of apneusis as is seen in the mammal when vagotomy is combined with a transection through the anterior pons: if facilitatory centres exist in the mesencephalon, they do not appear to be very potent.

The afferent pathways of the inhibitory reflex

The contrast between the predominantly inhibitory response elicited by tetanizing a branchial branch of the vagus and the complex intermixture of inhibitory and excitatory responses that results from tetanizing the cornea or an ophthalmic nerve has already been described. Using the response to a tetanic stimulation as a criterion, it has been possible to assess the extent to which the different motor nerves to the
pharynx and jaws carry inhibitory afferent fibres. All the four branchial branches of the vagus and the branchial branches of the IXth nerve produce complete inhibition like that depicted in Fig. 2E. The pre-spiracular branch of the VIIth was also equally potent in inhibition, a result that is of interest in that the spiracle though devoid of gill lamellae in the dogfish is generally considered to be a modified gill slit. The palatine branch of the VIIth was moderately effective, but respiration broke through after 3-4 sec.; no other branch of the VIIth was effective, nor were the maxillary and mandibular branches of the Vth.

![Fig. 6. The effect of a repeated single stimulus to the central end of a branchial branch of vagus when all the branchial branches of IXth and Xth nerves have been cut. A, without stimuli; B, with stimuli. Time, sec. Marks = single stimuli.]

**DISCUSSION**

The inhibitory reflex described here bears a resemblance to the Hering-Breuer reflex of mammals. In both, inflation excites receptors which discharge up the vagus and inhibit inspiration. Evidence from comparative anatomy and embryology (Goodrich, 1930) is held to point to an origin of the lungs of tetrapods from a posterior pair of gill pouches, and it would seem that a reflex mechanism elaborated to subserve branchial respiration has survived these evolutionary changes and has been utilized in the reflex control of respiration in mammals. That inhibitory afferents in the dogfish run in the branchial branches of the VIIth, IXth and Xth nerves reflects the fact that a fish retains more of the primitive metameric organization of the respiratory system than does a mammal. Since the lungs of tetrapods develop from a single pair of pharyngeal rudiments, it is not surprising that afferent fibres running from them travel only in one pair of nerves.

A further point of interest lies in the response to vagotomy. In the intact or decerebrate mammal this results in a slowing of respiration. The primary respiratory centre responsible for the generation of respiratory rhythms is located in the medulla, and this is played upon by both facilitatory and inhibitory influences from the pontine and mesencephalic reticular formation. Part of the afferent drive
pouring in from the vagus serves to facilitate the inhibitory system, and it is the loss of this after vagotomy that causes the over-facilitation evinced in the deeper, slower, sighing type of respiration (Breckenridge & Hoff, 1950). By contrast, vagotomy in a mammal deprived of these facilitatory and inhibitory influences by a transection between the pons and medulla results in an acceleration of respiration indicating that the vagus exerts a simple inhibitory influence at this level (Kerr, Dunlop, Best & Mullner, 1954). Vagotomy in the intact dogfish caused an acceleration of respiration; the fish responded like a mammal deprived of the regulatory influences of the higher centres. Moreover, transection immediately above the medulla in dogfish had little effect other than to increase the sensitivity to inflation. In their relative independence of down-flowing facilitatory influence the medullary respiratory centres resemble other generators of motor rhythms. The spinal mammal can neither walk nor stand. The spinal dogfish swims incessantly (Steiner, 1885). In locomotion as in respiration the dogfish is less dependent on the facilitatory support of the higher centres.

SUMMARY

1. Inflation of the pharynx of a dogfish causes an inhibition of respiration manifested as a reduction in rate and amplitude.

2. Tetanic stimulation of the central end of a cut branchial nerve also inhibits respiration.

3. These inhibitory responses differ in their greater regularity and duration from the transient inhibition arising from stimulation elsewhere in the body.

4. Both normal respiration and inflation cause the discharge of receptors whose impulses pass up the vagus nerve. The pattern of firing of these receptors during an inflation corresponds to the pattern of inhibition.

5. Brief inflations are more effective in securing inhibition if they arrive at a time when the receptors are not being caused to fire by a normal inspiration.

6. Cutting the branchial branches of the IXth and Xth nerves eliminates the pause between successive respirations and increases the respiratory rate.

7. These pauses can be made to reappear by periodically stimulating the central end of a cut branchial nerve.

8. Section of the brain between the medulla and the mesencephalon increases the sensitivity to inflation.

9. Inhibitory afferents run in all branchial branches of the IXth and Xth nerves and in the pre-spiracular branch of the VIIth nerve.

10. It is suggested that in its response to vagotomy the dogfish resembles a medullary mammal.

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