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THE INITIATION OF DIVING APNOEA IN THE FROG, *RANA PIPIENS*

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SUMMARY

1. Diving apnoea in *Rana pipiens* was initiated by submerging the external nares. As the water level was raised above the frog, both buccal and lung pressure increased by an amount corresponding to the water head. During submergence the external nares remained closed, although the apnoeic period was punctuated by ventilation movements which moved gas between the lungs and buccal cavity.

2. Bilateral section of the ophthalmic nerves did not alter the normal pattern of ventilation in air, although it often resulted in the intake of water into the buccal cavity on submergence. Introduction of water into the buccal cavity, either naturally as in denervates or by injection through a catheter in intact frogs, triggered sustained electromyographical activity in some respiratory muscles.

3. Electroneurograms recorded from the cut peripheral end of an ophthalmic nerve showed that receptors in the external narial region were stimulated by movement of a water meniscus across them. Activity could also be recorded in the ophthalmic nerve in response to water flow past the submerged nares. Punctate stimulation of the narial region confirmed that these receptors were mechanosensitive.

4. Bilateral electrical stimulation of the central ends of cut ophthalmic nerves in lightly anaesthetized frogs caused apnoea with a latency of less than 200 ms. The external nares remained closed throughout the period of stimulation although buccal pressure events, resembling underwater ventilation movements, occurred when stimulation was prolonged.

INTRODUCTION

All air-breathing vertebrates become apnoeic on submersion in water in order to exclude water from the airways and gas exchange surfaces, but it is only recently that the mechanisms initiating and maintaining diving apnoea have become the subject of concerted investigation. At present there is a growing amount of evidence that, in birds and mammals at least, sensory information from the area around the nostrils, the nasal cavity, and in some cases the region of the glottis and upper respiratory tract, is involved in the respiratory adjustments to submersion (Andersen, 1963; Butler & Jones, 1968; Tchobroutsky, Merlet & Rey, 1969; Rey, 1971; Angell James & Daly, 1972*a*, *b*; Drummond & Jones, 1972; Dykes, 1974; Leitner & Roumy, 1974; Leitner,

Roumy & Miller, 1974; Bamford & Jones, 1974). It is likely that, in the majority of divers, more than one afferent pathway is involved. For instance, in ducks different peripheral regions innervated by the glottal and trigeminal nerves respectively have been shown to initiate apnoea with appropriate stimuli (Bamford & Jones, 1974). Although amphibians are more completely adapted to a semiaquatic existence than other diving vertebrates, the skin acting as a surface for gas exchange underwater, the airways must still be protected from water during immersion by the inhibition of ventilation. However, there is little information available about the stimulus which causes apnoea during periods of submergence in amphibia or the sites sensitive to such stimulation.

Willem (1920) thought that narial closure and apnoea on submersion in *Rana esculenta* were reflex, due to the wetting of the snout, and that the occasional release of lung gas underwater through the nares involved the temporary overriding of this reflex inhibition. Spurway & Haldane (1953) agreed with this view, considering that the presence of water at the snout provided an inhibitory stimulus to ventilation in newts and that the resumption of ventilation observed on surfacing of the snout was due to 'the cessation of inhibitory sensory stimuli rather than because of any positive sensory stimuli from the air'. The purpose of this investigation was to determine the sensory areas important in the initiation and maintenance of diving apnoea in the frog *Rana pipiens* and to investigate the effects of denervation of these areas; to record from the appropriate sensory nerves during simulated dives; and finally to attempt to initiate apnoea in frogs by electrical stimulation of these nerves, in order to establish their role during diving.

METHODS

Experiments were performed on 65 *Rana pipiens* of weights ranging from 30 to 85 g, although animals of 75–85 g were used exclusively in the nerve stimulation experiments. All the experiments were performed at an air temperature of 24 °C and at a water temperature of 20 °C.

Experiments involving submergence of intact animals and animals whose ophthalmic nerves had been sectioned, were carried out in a Perspex tank of 3 l capacity. The tank was connected to a large water reservoir so that the water level could be raised and lowered at will. The animals were positioned on a cork board and restrained by pinning (Jones, 1970). During normal dives, buccal and lung pressures were recorded with Hewlett-Packard 268 BC pressure transducers, and buccal volume changes were recorded by a photocell as described previously (West & Jones, 1975). Following ophthalmic nerve section, frogs often struggled during submergence which rendered these methods impractical, and ventilation was monitored by recording EMG activity from the posterior intermandibular muscle and the laryngeal dilator muscles.

The ophthalmic nerves were sectioned under deep MS 222 anaesthesia induced by placing the animal in a solution containing 500 mg/l. The frog was placed on its back, the jaws were held apart, and incisions were made in the floor of the nasal cavity from the internal narial openings to the midline. The cartilages forming the floor were then reflected back to the midline, exposing the nasal cavities. The ophthalmic nerves enter each nasal cavity through foramina in the sphenethmoid cartilage, and then branch across the cavities, between the cartilage and the olfactory epithelium. The branches

pass through the skull to supply the skin of the external narial region and the snout (Ecker, 1889). The nerves were cut bilaterally, close to the point of emergence from the sphenethmoid cartilage, while for sham operations they were merely identified through slits made in the olfactory epithelium parallel to their courses but were not sectioned. The nasal cartilages were then returned to position and, in the larger frogs, held in place by silk stitches. Both denervated and sham-operated animals were allowed to recover from the anaesthesia and left for 24 h in a large holding tank before being used in an experiment. Only those frogs which appeared to breathe normally after the operation were used.

Recordings of afferent nervous activity were made from branches of the ophthalmic nerves at the level of the nasal cavity. The nerves were sectioned centrally in double-pithed frogs and placed on a pair of fine, silver-wire hook electrodes under mineral oil. Those branches with their receptive fields around the external nares were chosen for the recordings. The response to a water meniscus moving over the nares and to water flow was investigated by placing the frogs in the 3 l tank, in which the water level could be raised and lowered. The frogs were pinned on their backs with the lower jaw held back, and the external narial openings were plugged to prevent water entering them and thus grounding the electrodes. To determine the effects of pressure, some trials were carried out in which the narial region was submerged under 4–6 mm of mercury, retained around the nares by a modelling clay dam, or with the entire frog submerged under mineral oil (sp.gr. 0·87) to depths of approximately 5 cm.

Recordings were made from the cutaneous branches of the second, third and fourth spinal nerves in order to determine the responses of cutaneous receptors serving other areas of the skin to pressure and to the movement of a water meniscus. In order to approximate the conditions at the snout, where the skin is firmly connected to the underlying cartilage, flaps of skin containing the receptive fields were placed on a ground-glass disc to which hydrostatic pressure was applied by means of a water column. The fibres serving a receptive field were led out through a small hole in the centre of the disc and placed over hook electrodes under mineral oil.

Nervous activity was amplified and filtered by means of a Tektronix 122 pre-amplifier and displayed on a Tektronix 502 A oscilloscope, being simultaneously stored on a Hewlett-Packard 3900 C tape-recorder. Suitable signals were later photographed on playback by a Grass oscilloscope camera, or played into a Brush pen-recorder at one-quarter recording speed. In some experiments a ratemeter was used to determine firing rate.

In experiments involving electrical stimulation of the ophthalmic branch of cranial nerve V, the nerves were exposed bilaterally at the level of the orbit. It was necessary to remove the eyes under deep (500 mg/l) MS 222 anaesthesia in order to expose the nerves. Initially the nictitating membrane was removed and the eye muscles were cut close to the eyeball, and the optic nerve, artery and vein were ligatured. The optic stalk was then cut distally to the ligature and the eye removed. The ophthalmic branch of V, which runs between the cranium and the eyeball, below the superior rectus muscle but above all the other eye muscles, was then carefully freed from its connective tissue sheath and associated blood vessels. Before recovery from the anaesthesia the frogs were secured in a head holder, which rigidly fixed the head in relation to the stimulating electrodes, but allowed ventilation movements to occur normally (Jones, 1970).

Ventilation restarted spontaneously as the animal recovered from the plane of surgical anaesthesia. By application of MS 222 solution to the skin (200 mg/l), complete recovery was prevented and an anaesthetic level was maintained which still allowed normal ventilation movements to occur. Before the frog had resumed ventilation the nerves were placed on silver/silver-chloride bipolar stimulating electrodes under mineral oil, and the distal ends of the nerves were cut at the anterior end of the orbit. Fine hook electrodes were used, the stimulating pulses being provided from a Grass model S4G stimulator, and displayed on a Tetronix 502 A oscilloscope. Unipolar stimulation was used at 50–500 Hz, 0·4–4 msec duration and 30 mV to 5 V intensity. The low stimulating voltages used seemed to preclude the possibility of stimulus spread, and care was taken that the electrodes only contacted the ophthalmic nerve. Ventilation movements were monitored by recording buccal pressure, which was displayed on a Hewlett-Packard four-channel pen-recorder, while periods of nerve stimulation were indicated by means of the event channel. Care was taken to keep the skin of the animals moist throughout the course of these experiments.

RESULTS

(*a*) *Preliminary experiments*

Several types of preliminary experiments were performed on *Rana pipiens* to determine the site initiating apnoea on immersion in water. In the first of these, as the water level was gradually raised the effects on ventilation were noted. In 27 out of a total of 30 trials on 5 frogs, the nares closed and ventilation movements ceased when the water reached the level of the external nares. In three experiments there was a brief period of apnoea when water came in contact with the buccal floor, but normal ventilation movements were resumed after 10–15 s and continued until the water had risen to the level of the external nares.

When the frogs were surfaced after periods of submergence which varied from 2 to 15 min, resumption of breathing occurred in 23 cases immediately the water level fell below the level of the external nares. In three trials breathing did not resume until after a lag of 10–30 s, while in the remaining four trials breathing did not restart until the water level had fallen below the level of the buccal floor. Three frogs, blinded by section of the optic nerves, performed in the same way as the intact animals. Furthermore, no variations in ventilation rate could be induced in frogs which were placed in a large beaker in a tank in which the water level was raised and lowered. In these experiments the water surface passed across the frogs' visual field, although the frogs never came into contact with the water.

It was possible, however, that the presence of water on the surface of the eyes or tympanic membranes, both on a level with the external nares when the frog is in a natural resting position, could be important in the development of apnoea. To test this hypothesis 20 trials were made with three frogs in which the frogs were secured to a vertical cork block, head up, so that the tympanic membranes, tympanic memranes and eyes, and finally the tympanic membranes, eyes and external nares could be submerged by raising the water level. In no trial could apnoea be induced by wetting the tympanic membranes, or the tympanic membranes and eyes; only when the water meniscus was at the level of the external nares did narial closure and apnoea occur.

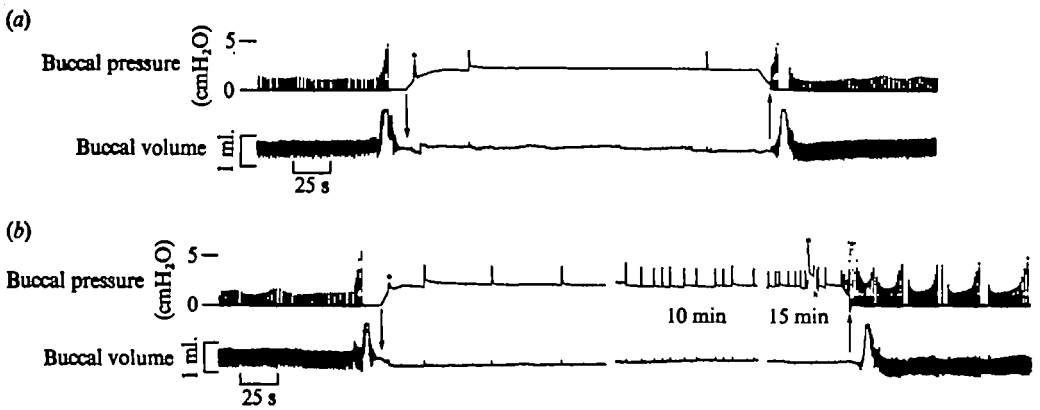

Fig. 1. Buccal pressure and volume changes recorded from unanaesthetized *Rana pipiens* before during and after a 4 min dive (*a*) and a 15 min dive (*b*). Upper trace in (*a*) and (*b*), buccal pressure; lower-trace, buccal volume. Increase in buccal volume is up on trace. Arrows indicate time at which water surface crosses the nares on immersion and surfacing. Baseline changes in the volume trace on immersion and surfacing are an artifact caused by the water surface moving across face of photocell. ●, Gas bubble release from nares. In both (*a*) and (*b*) narial closure occurs during a sequence of buccal ventilation cycles.

These preliminary trials strongly suggested that water at the level of the external nares induced apnoea in *Rana pipiens*.

(*b*) *The effect of diving on lung pressure, buccal cavity pressure and volume in normal animals*

Fig 1 (*a*) and (*b*) illustrates the pressure and volume changes recorded from the buccal cavity of frogs during the course of two dives of differing duration. The water surface reached the level of the external nares in 10–20 s in each case, at which point the nares closed and respiratory movements ceased. In both cases the nares closed during a sequence of low-amplitude buccal ventilation cycles when the lungs were inflated and isolated by the closed glottis from the buccal cavity (West & Jones, 1975). A few seconds after narial immersion bubbles of gas were released from the nares, bubble release being preceded by a rapid increase in buccal pressure (Fig. 1 *a*, *b*). In cases where animals were submerged with the lungs full, this gas represented part of the lung contents. In others, it represented loss of some of the buccal gas (Fig. 2 *a*) and resulted in the buccal floor being closely opposed to the roof of the buccal cavity during the dive due to its reduced gas volume. In the early part of the period of submersion both lung and buccal pressure slowly increased in response to the increase in hydrostatic pressure as the water rose, until both had increased by 2–3 cmH_2O which corresponded to the height of the water column above the frog.

Throughout the course of a dive, periodic pressure events similar to lung ventilation cycles were recorded from the buccal cavity and lungs (Figs 1 *a*, *b*, 2 *a*, *b*). Glottal opening resulted in a simultaneous fall in lung pressure and increase in buccal pressure, followed by equilibration of lung and buccal pressures. After equilibration, decrease in buccal volume raised the pressure in the system until the original lung pressure was attained, at which point the glottis closed, isolating the lungs once more.

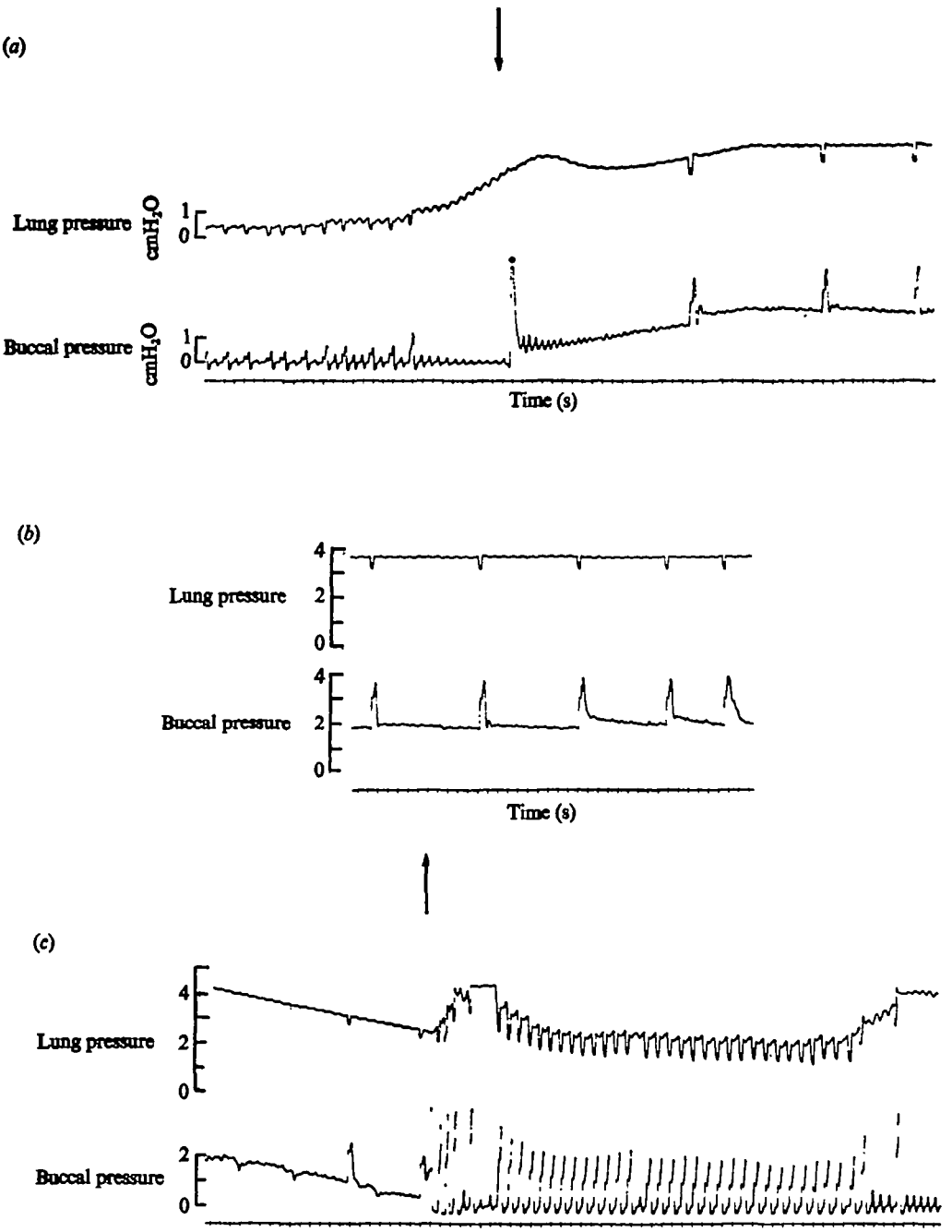

Fig. 2. Lung and buccal pressure changes recorded from unanaesthetized, restrained *Rana pipiens* showing (*a*) initiation of apnoea on narial immersion, (*b*) 'underwater' pattern of ventilation 10 min after immersion, (*c*) hyperpnoea which occurs on surfacing. In (*a*) and (*c*) arrow indicates narial immersion and surfacing respectively; upper trace, lung pressure; lower trace, buccal pressure. Time marker (at bottom) = 1 *s*. ●, Bubble release through nares.

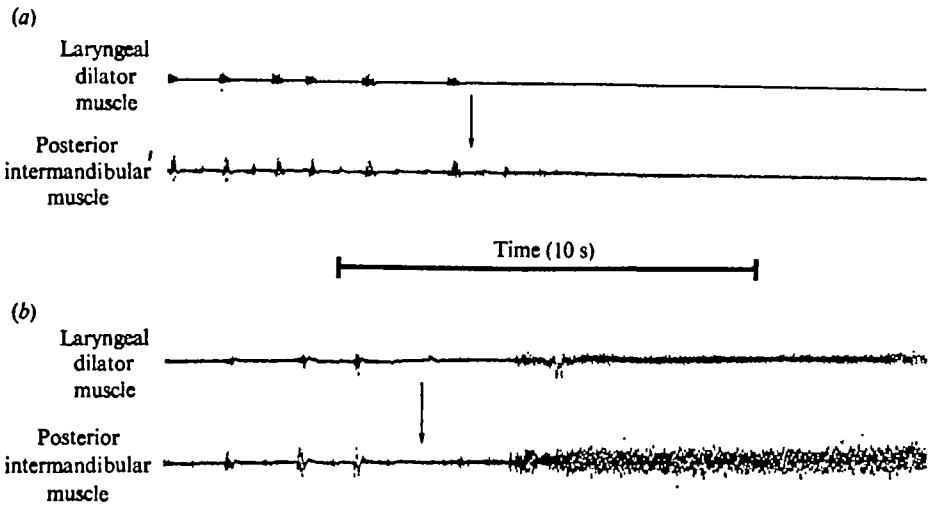

Fig. 3. E.M.G. activity from laryngeal dilator and posterior intermandibular muscles before and during a dive in an intact frog (*a*) and a frog in which the ophthalmic nerves had been bilaterally sectioned (*b*). Upper trace of each pair, activity recorded from laryngeal dilator muscle. Lower trace, activity recorded from posterior intermandibular muscle. Downward pointing arrows in (*a*) and (*b*) indicate submersion. The horizontal bar between the pairs of traces indicates 10 sec for both (*a*) and (*b*).

Buccal volume and pressure then returned to their previous levels. The posterior intermandibular muscle of the buccal floor was active during this phase, as presumably were the other muscles involved in lung ventilation cycles. The frequency of underwater lung ventilation cycles was very variable, ranging from 1 to 10/min in individual frogs. Frequency often increased during the course of a period of submergence (Fig. 1*b*). Lung pressure was usually maintained at a constant level throughout the period of submergence, although buccal pressure often fell slightly. During longer dives there was also a slight increase in buccal volume (Fig. 1*b*), suggesting that buccal pressure may have initially been held slightly higher than the hydrostatic pressure by tone in the muscles of the buccal floor. The period of apnoea caused by submersion ended when the water surface fell past the level of the external nares. Emersion initiated a burst of lung ventilation cycles of high pressure, followed by an increased frequency of ventilation (Fig. 2*c*), most of the cycles being of the lung ventilation type.

(*c*) *Effect of ophthalmic nerve section on initiation of and recovery from diving apnoea*

The regular rhythmic electrical activity that is associated with air ventilation and can be recorded from the laryngeal dilator muscles and the posterior intermandibular muscle of the buccal floor stopped immediately when water covered the external nares in normal and sham-operated frogs (Fig. 3*a*) and restarted after narial emergence. Bilateral section of the ophthalmic nerves at the level of the nasal cavity resulted in an altered response to submergence and emergence. Instead of a cessation of electrical activity, sustained tonic activity replaced the rhythmic activity in both the posterior intermandibular muscles and the laryngeal dilator muscles 1–2 s after narial submergence. In view of the function of the laryngeal dilators it is difficult to account for

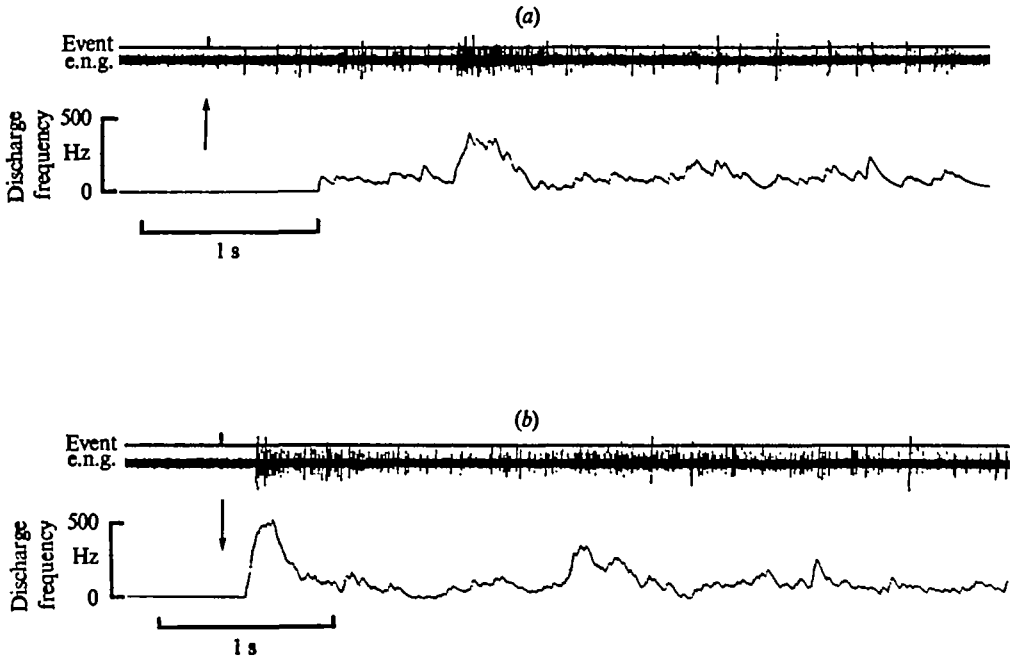

Fig. 4. Response of a narial branch of the ophthalmic nerve to the passage of a water meniscus across the nares. Top trace, event marker; middle trace, neurogram; lower trace, frequency of total neural activity. (*a*) at arrow, meniscus was raised across external nares. (*b*), at arrow meniscus was lowered from the external nares. In this experiment the animal was on its back so that the water passed, with respect to the external nares, in a direction which would usually be associated with emergence in (*a*) and submersion in (*b*).

the tonic activity detected by the electrodes in this muscle, although it is possible that under these conditions it consisted of cross-talk from the intensely tonically active intermandibular muscle (Fig. 3*b*). During this activity in the respiratory muscles the frog struggled, and the buccal floor was raised, acquiring an uncharacteristic flattened appearance. At the same time, gas was lost through the nares, and the jaws sometimes gaped. The tonic electrical activity from the muscles died down in 1–2 min and the frogs became quiescent. Narial emergence did not initiate ventilation in these denervated frogs. Ventilation restarted spontaneously in two of the five frogs in the second and third minute respectively after emergence, while in the remaining three it had not resumed by the fifth minute. Water was found in the buccal cavities and lungs of these three frogs *post mortem*, and also in the buccal cavity of one of the two frogs which resumed ventilation spontaneously. Injection of 0·2 ml of water into the buccal cavity via a cannula inserted through the tympanic membrane in intact frogs, either during submergence or when in air, produced similar responses with respect to recorded muscle activity and struggling behaviour to those observed in the denervates.

(d) Electroneurograms recorded from the ophthalmic nerve in response to static and dynamic water pressures and mechanical stimulation

When the external nares were submerged in water at 20 °C, spike activity in branches of the ophthalmic nerve serving the skin around the external nares was initiated.

Activity was greatest during the time when the water surface was moving over the external nares, and gradually adapted afterwards (Fig. 4*a*). Emersion of the nares resulted in a similar burst of spike activity, the units involved being those of large and intermediate spike height (Fig. 4*b*). The closeness of the recording sites to the nares limited the depth of water that could be used to 5–6 mm, and under these conditions complete adaptation to continued submergence occurred in approximately 5 s and in 10 s after emergence. Once adaptation to submergence had occurred, activity in the receptor population could be restarted by causing water flow over the nares. In order to simulate the effect of pressure at greater depths mercury or mineral oil was used. The response obtained when a mineral oil meniscus moved across the external nares was usually not as great as the response obtained with a water meniscus, possibly because of the lower density of the oil (sp.gr. 0·87). The nerves fired for longer periods at the higher stimulating pressures made possible by using mineral oil or mercury; for example, in a typical trial using mercury, impulse traffic at 20 Hz was recorded after 4 min exposure to a simulated depth of 5·6 cmH_2O. All the units involved in these responses were apparently mechanoreceptors since they all responded to punctate stimulation of their receptive fields around the nares.

Receptors serving the cutaneous branches of the second, third and fourth spinal nerves responded to suddenly applied punctate stimulation of their receptive fields in a similar way to those served by the ophthalmic nerve, but no response to the passage of a water meniscus was obtained in 12 preparations. Response to an increase in hydrostatic pressures to 10 cmH_2O in 2 cmH_2O increments, followed by a sudden fall to atmospheric pressure was limited to a few fibres responding to the initial pressure increment and to the fall in pressure, with no increase in tonic activity at any pressure level, nor any response to the passage of the water meniscus.

(*e*) *The effect of stimulating the ophthalmic nerves on initiation and maintenance of apnoea*

Bilateral electrical stimulation of the central ends of the cut ophthalmic nerves at the level of the orbit initiated periods of apnoea with a latency of less than 200 ms. The most effective frequency of stimulation was from 250–500 Hz, while the threshold voltage required to cause a period of apnoea varied from 30 to 300 mV in individual frogs, with pulse duration varying from 1–4 ms. Frogs in apnoea became very quiescent and virtually no struggling occurred. The apnoeic periods did not continue indefinitely. Stimulation at a voltage just above the threshold voltage produced short periods of apnoea of 10–20 s duration which were terminated by the resumption of ventilation, even though stimulation still continued. The nares remained open in these short periods of apnoea, and buccal pressure remained at atmospheric. A slight increase in the stimulating voltage caused the nares to close at the start of the period of apnoea and buccal pressure to be held slightly above atmospheric until the nares opened once more, often midway through the apnoeic period. Further increase in the stimulating voltage produced longer periods of apnoea with closed nares and, in many cases, narial closure was maintained throughout the period of stimulation (25 min). On narial closure, buccal pressure was initially maintained above atmospheric, presumably due to tone in the muscles of the buccal floor, although it fell slowly during the apnoeic period (Fig. 5). Buccal volume was small during apnoeic periods, the buccal floor being elevated, and often small pressure fluctuations reflecting the heartbeat

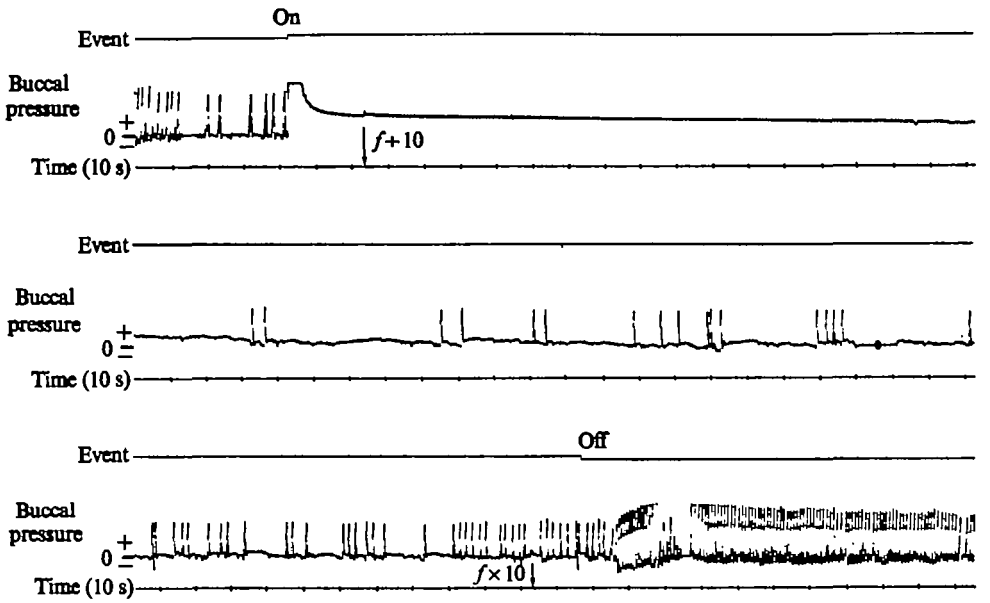

Fig. 5. The effect on breathing movements of bilateral electrical stimulation of the cut central ends of the ophthalmic nerves. The frog was in air throughout. The three sets of traces are continuous and the upper trace is the event marker (upward deflexion indicates start of stimulation and downward deflexion shows end of the period of stimulation); middle trace, ventilation as indicated by buccal pressure; lower trace, time (10 s). At the start of stimulation the pulse parameters were 100 Hz, 300 mV amplitude, 4 ms duration; pulse frequency was reduced by an order of magnitude at the first arrow (upper set of traces) and increased to the initial rate at the second arrow (lowest set of traces).

were present in the buccal pressure trace. In an attempt to simulate the situation during a normal dive, where presumably impulse frequency in the trigeminal nerve peaks on submersion and emersion while adaptation occurs during the dive, in some trials the stimulating frequency was reduced by an order of magnitude shortly after the initiation of apnoea and was then briefly pulsed at the original frequency just before the end of stimulation (Fig. 5). This method proved to be as effective in producing apnoea as maintaining the original frequency of stimulation throughout. Pressure events resembling underwater lung ventilation cycles occurred with increasing frequency throughout the longer periods of apnoea (Fig. 5). Narial opening did not occur during these cycles so that if buccal pressure was initially above atmospheric it never fell to the atmospheric value. These cycles appeared similar in every respect to those occurring during submersion.

Narial opening and the resumption of normal ventilation cycles at the end of a stimulation period occurred after a lag of about 10 sec (Fig. 5). Even after periods of apnoea as short as 3–5 min the frequency of buccal breathing movements was greater than the pre-apnoeic frequency, and breathing initially consisted entirely of lung ventilation cycles. Efforts were also made to simulate the effects of water flow past the external nares by gating the stimulating pulses so that their frequency varied randomly between zero and the maximum frequency of stimulation throughout the stimulation period. This method, however, proved less effective in producing apnoea than the two methods described in detail above.

To test whether sites other than the external nares were important for the initiation and maintenance of apnoea, the duration of apnoea provoked by bilateral stimulation of the ophthalmic nerves when the frog was submerged to the level of the external nares was compared with that induced when the frog was completely out of water. No consistent difference in the length of the apnoeic period was discernible in the two conditions. However, stimulation of one ophthalmic nerve was consistently less effective in maintaining apnoea than bilateral stimulation. Bilateral stimulation of the cutaneous branches of the dorsal branches of the second, third and fourth spinal nerves performed as a control produced no changes in the respiratory pattern until the voltage was increased to a point where struggling occurred. Bilateral stimulation of the abdominal cutaneous branch of the third spinal nerve with 1–2 V, 250 Hz, 3 msec pulses inhibited lung ventilation cycles in two of five frogs, although the ventilation cycles reappeared before the end of the period of stimulation. Sâto (1954) produced a similar effect in *Rana nigromaculata* by lightly clipping the abdomen.

DISCUSSION

The results indicate that submergence of the external nares is an adequate stimulus for the initiation of diving apnoea in *Rana pipiens* and that water does not normally enter the nasal or buccal cavities. The nares are closed during a dive, but occasional respiratory movements occur in which gas is moved from the lungs to the buccal cavity and back again. Bilateral section of the ophthalmic nerves, whose sensory fibres serve the narial region and the snout, resulted in a failure to close the nares on submergence and the entry of water into the buccal cavity, and in some cases the lungs. Muscles necessary for narial closure may be deprived of motor innervation by section of the ophthalmic nerves; however, no efferent activity could be recorded from the central ends of cut ophthalmic nerves, although it is possible to record E.M.G. activity synchronous with narial closure from the region of the external nares (Jones, 1970; West & Jones, 1975). Presumably the motor innervation to narial muscles is via the maxillary branch of V; this is perhaps confirmed by the fact that narial closure occurred during bilateral stimulation of the cut central ends of the ophthalmic nerves, and that frogs with severed ophthalmic nerves are capable of generating normal lung ventilation cycles, which require effective narial closure (Fig. 5). It has also been shown that the indirect action of the lower jaw muscles on the premaxilla is effective in narial closure in *Rana catesbeiana* (De Jongh & Gans, 1969).

Entry of water into the buccal cavity caused sustained tonic activity in the posterior intermandibular muscle of the buccal floor, and probably other buccal floor muscles, as well as struggling on the part of the frog. Zotterman (1949) suggested that the tongue 'water receptor' of the frog might reflexly contribute in keeping the mouth of the frog closed as well as inhibiting the respiratory movements when under water. Although the primary receptive site is the area around the external nares and water does not normally enter the mouth on submersion, it seems likely that sensory information from the tongue is responsible for the responses which are observed in denervated frogs and appear to be designed to clear the buccal cavity of water by reducing buccal volume.

Recordings made from the ophthalmic branch of the trigeminal nerve show that

skin mechanoreceptors in the region of the external nares are able to respond to the movement of a water meniscus across their receptive fields in a simulated dive. Gregory (1973), working on ducks, could find no response to simulated diving from beak mechanoreceptors served by the ophthalmic nerve. In the units he investigated there was no response even when the hydrostatic pressure at the surface of the beak was raised and lowered between 0 and 50 cmH_2O, although he points out that they may not have been the most sensitive units present. The results of Leitner & Roumy (1974) suggest that beak thermoreceptors are more important than mechanoreceptors in causing apnoea in the duck; immersion in 15 °C water causes immediate apnoea, whereas there is a 10 s lag if 37 °C water is used. Bamford & Jones (1974) on the other hand suggest that cold-sensitized slowly or non-adapting receptors in the glottis may also be important in initiating apnoea in ducks. Nevertheless, thermoreceptive information would appear to be of little value in the frog, a poikilotherm, where the body temperature would be close to that of the surrounding water. The receptive units involved in the frog appear to be mechanoreceptors and similar to Catton's (1958) type *a* and *b* fibres, as they produce respectively large fast adapting spikes and smaller, relatively slowly adapting spikes with a lower threshold. According to Catton (1958), these spikes are propagated in myelinated fibres, while the receptors appear to be free nerve endings. The snouts of two frogs were serially sectioned and stained in Glee's silver stain, but no specialized endings could be found in the region of the external nares.

In most preparations, mechanoreceptor response to a stimulus of a few cmH_2O lasted a matter of minutes. If trigeminal input is an important factor in inhibiting rhythmic activity in the respiratory centre during immersion by, for example, overriding chemoreceptive input, it seems necessary that it should be maintained throughout the dive. How then do frogs maintain apnoea in dives lasting 1 h or more? It is feasible that, in the field, movements of the frog, or currents in the body of water, could bring about continuing neural activity in response to flow. Furthermore, increase in pressure due to deep diving would provide a greater stimulus intensity than those investigated, possibly causing the recruitment of less-sensitive units. However, frogs immobilized on boards and submerged in a few centimetres of water for periods of 1 h maintain apnoea and spontaneously resume ventilation upon emergence (Jones, 1967). It is possible that the trigeminal mechanoreceptors in intact animals could become sensitized during the course of a dive, enhancing their afferent input to the C.N.S. It has been demonstrated in *Rana pipiens* that stimulation of the first sympathetic ganglion results in a sympathetic efferent response in cutaneous branches of the trigeminal nerve, which elicits an afferent mechanoreceptor discharge (Chernetski, 1964*a*). The possibility of such a sympathetic enhancement of trigeminal receptor input on immersion does not seem unreasonable in frogs, where there are close morphological relations between the cranial nerves and the sympathetic system (Chernetski, 1964*b*), although it was obviously not observable in the double-pithed preparations used in these experiments.

From the above evidence it appears likely that electrical stimulation of the ophthalmic nerves at the orbit mimicked the effect of mechanoreceptor input from the region of the external nares and snout. Stimulation near the threshold voltage resulted in brief periods of apnoea during which the external nares remained open and buccal pressure

was atmospheric, whereas increase in voltage induced longer periods of apnoea with closed nares. Electrical, mechanical or chemical stimulation of the nose is known to cause reflex apnoea and bradycardia in several mammalian species (Brodie & Russel, 1900; Lombroso, 1913; Allen, 1928). More recently, Angell James & Daly (1972*a*) produced periods of apnoea of 10–40 s duration in dogs by drawing tap water or saline solution over the nasal mucosa. The liquid had to be in motion to initiate apnoea, suggesting that information from nasal mechanoreceptors, probably bare nerve endings, was of prime importance.

Hyperpnoea occurred after short dives and also after short periods of apnoea induced by trigeminal stimulation. In mammals, hyperpnoea is probably a response to the increase in arterial P_{CO_2} which occurs in the apnoeic period (Angell James & Daly, 1969), while in the frog the fall in blood pressure that occurs during long periods of submersion may also help stimulate hyperpnoea on surfacing (Jones, 1967). De Marneffe-Foulon (1962) found that endopulmonary pressure could control the ventilation rate in the frog, a fall in pressure stimulating ventilation. It has been suggested that this may explain post-dive hyperpnoea if lung pressure falls on submersion due to the release of gas bubbles at the start of the dive (Jones, 1966). However, lung pressure invariably rises when a frog is submerged even though lung volume may be low, the lungs acting as simple closed hydrostats. Although nothing is known of the central mechanisms involved, it seems more likely that the increase in ventilatory drive after short periods of apnoea in air is a rebound effect due to the absence of rhythmic input from lung mechanoreceptors during the apnoeic period and to the direct influence of the release of trigeminal inhibition on the respiratory centre.

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