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THE INITIATION AND MAINTENANCE OF BRADYCARDIA IN A DIVING MAMMAL, THE MUSKRAT, *ONDATRA ZIBETHICA*

BY P. C. DRUMMOND AND DAVID R. JONES

From the Zoology Department, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

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SUMMARY

1. The cardiac and respiratory responses shown by muskrats in both unrestrained and restrained dives have been compared with responses elicited by stimulation of a number of cardio-depressant receptor inputs, in an attempt to determine which are most important in initiating and maintaining diving bradycardia.

2. In unrestrained voluntary dives heart rate fell from 310 ± 3 to 54 ± 3 beats min^{-1} in 1 to 2 sec, which was significantly below that seen in dives by restrained unanaesthetized or anaesthetized animals.

3. Pouring water on the external nares during maintained artificial ventilation caused heart rate to decline to 76 ± 12 beats min^{-1} after 1 sec. Flowing water through the internal nares caused apnoea, in the expiratory position, and bradycardia within one third of a second. Heart rate fell to 20 ± 2 beats min^{-1}, 1 sec after the start of water flow. Substituting saline for water reduced both the apnoeic and cardiac responses. Bilateral section of the maxillary branch of V and the inferior laryngeal (X) nerves completely abolished the cardiac and respiratory response to water flow.

4. Artificial ventilation throughout periods of nasal stimulation with water or saline reduced the bradycardia, although even the saline driven response could not be completely abolished. Lung deafferentation eliminated any direct effect of artificial ventilation on heart rate during nasal stimulation.

5. Lung deflation caused bradycardia within $0{\cdot}97 \pm 0{\cdot}17$ sec, heart rate falling from 268 ± 7 to 59 ± 4 beats min^{-1}. Bradycardia also occurred during maintained lung inflation but it was delayed for a period which varied from $6{\cdot}8 \pm 1{\cdot}8$ sec at an inflation pressure of 0·5 kPa to 35 ± 7 sec at 1·5 kPa.

6. Bradycardia caused by nasal water flow or lung deflation was unaffected by bilateral section of the sinus nerve.

7. Artificial ventilation of paralysed muskrats with 5% CO_2 in N_2 caused bradycardia when P_{a,O_2} reached $8{\cdot}4 \pm 0{\cdot}8$ kPa and heart rate declined to 76 ± 7 beats min^{-1} at 4 kPa. Bilateral section of the sinus nerve delayed bradycardia until P_{a,O_2} reached $4{\cdot}5 \pm 0{\cdot}5$ kPa.

8. These results suggest that the cardiac response to submergence could be the expression of input from three groups of receptors, nasal, lung and carotid chemoreceptors, although it is not clear how they interact with one another to generate the cardiac responses displayed by unrestrained animals during submergence.

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INTRODUCTION

The initiation and maintenance of bradycardia in diving birds and mammals during submergence has been the subject of intensive investigation in recent years (see Angell-James & Daly, 1972; Jones & West, 1978). In some species the cardiac response to forced submergence differs markedly from that displayed in the wild (Butler & Woakes, 1976) while in others the response is essentially similar although somewhat labile (Elsner, 1965, 1969; Murrish, 1970; Jones, Fisher, McTaggart & West, 1973). In prolonged restrained dives it has been shown repeatedly, for both birds and mammals, that arterial chemoreceptors make an important contribution to the cardiac adjustments (Hollenberg & Uvnäs, 1963; Angell-James & Daly, 1969; Jones & Purves, 1970; Daly, Elsner & Angell-James, 1977; Elsner, Angell-James & Daly, 1977). However, in many diving mammals bradycardia is initiated before there are any marked changes in blood gas content or pH. This bradycardia results from noxious stimulation of the internal or external narial region (Angell-James & Daly, 1972; Drummond & Jones, 1972; Elsner *et al.* 1977; Daly, Korner, Angell-James & Oliver, 1978). Furthermore, it is claimed that nasal receptor input enhances the depressor effects of the arterial chemoreceptors (Angell-James & Daly, 1973; Strømme & Blix, 1976; Elsner *et al.* 1977). Input from lung receptors or the activities of central respiratory neurones are seen as having a mcdifying effect on these responses in that they no longer stimulate the cardiovascular system and therefore allow full expression of depressor chemoreceptor and nasal reflexes (Anrep, Pascual & Rössler, 1936*a*, *b*; Daly & Scott, 1958; Daly & Hazzledine, 1963; Angell-James & Daly, 1973; Bamford & Jones, 1976; Lopes & Palmer, 1976; Angell-James & Daly, 1978). The role of arterial mechanoreceptors in effecting diving bradycardia in birds and mammals is controversial. In birds, selective denervation of aortic baroreceptors has no significant effect on diving bradycardia (Jones, 1973; Jones & West, 1978) yet, even so, more recent work has claimed that diving bradycardia results from a barostatic reflex activated by the incipient rise in blood pressure caused by chemoreceptor induced peripheral vasoconstriction (Andersen & Blix, 1974; Blix, Gautvik & Refsum, 1974). Baroreceptors contribute to the bradycardia of primarily nasal origin in the rabbit (White & McRitchie, 1973) while in diving mammals their role has not been elucidated.

The purpose of the present investigation was to elicit as many of these cardio-depressant reflex pathways as possible in an accomplished mammalian diver, the muskrat (*Ondatra zibethica*), to establish which are most important in initiating and maintaining bradycardia during submergence. Muskrats are easily obtained and managed in the laboratory and diving bradycardia appears to be unaffected by anaesthesia (Drummond & Jones, 1972). For these reasons, we undertook this preliminary study in the hope that in future experiments it would prove possible to use muskrats to elucidate central neural integration of cardio-depressor reflexes.

METHODS

The experiments have been done on 128 juvenile and adult muskrats varying in weight from 0·7 to 1·3 kg. All the animals were trapped in the vicinity of Delta, B.C., Canada and were held, in pairs, in pens at the Vivarium of the University of British Columbia. Each pen (3 m × 1 m) consisted of a pond 0·5 m deep which was covered at one end by a plywood platform for nesting.

The animals were able to move freely from water to the nesting area. The animals were fed apples, carrots, and lettuce and the diet was supplemented with high protein rabbit 'chow' (Purina, St Louis, Mo., U.S.A.).

The adjective 'initial' when referring to any of the measured variables describes them before submergence. The adjectives 'intact' and 'normal' refer to animals which did not undergo any nerve section. The adjective 'control' refers to the value of any measured variable before any experimental intervention. In text and Figures numerical values, when referring to determinations of variables in a group of animals, are given as means ± S.E. of the mean of n determinations. In data analysis bradycardia was recognized when the cardiac interval, after a given intervention, lengthened by more than 10 % from its value in the control period.

(1) *Operative procedures and recording techniques*

All operative procedures were performed under general anaesthesia (urethane, 950–1350 mg/kg or Nembutal, 60 mg/kg). The anaesthetics were injected I.P. after first sedating the animal with ether. Approximately half the experiments were done on animals paralysed by injection of curare (D-tubocurarine chloride, 2 mg/kg I.P.) and in the later stages of these experiments areas of incision were periodically infiltrated with local anaesthetic (Xylocaine, 2 %). For studying diving under natural conditions or responses to forced submergence we used unanaesthetized animals in which wires for recording the electrocardiogram were implanted under Nembutal anaesthesia. Thalamic decerebration was done under urethane anaesthesia. The fur was shaved from the skull and the skin divided by a mid line incision. Using a dental burr, a hole (1 × 1·5 cm) was cut in the parietal bone. The bone was removed and the dura slit with the point of a hypodermic needle and reflected. The cerebral hemispheres were removed by aspiration and bleeding was controlled by cautery.

(*a*) *Arterial blood pressure and gas measurement.* Arterial blood pressure and P_{a,O_2} were measured from a cannula in the carotid artery, usually on the left side. With the animal on its back the trachea was exposed after opening the neck with a mid line incision. The carotid artery was located running alongside the trachea and cannulated with a polyethylene cannula which was attached to a Harvard 377 (Harvard Apparatus, Millis, Mass. U.S.A.) pressure transducer or a flow through cuvette containing an oxygen electrode. In the latter case, blood flowed continuously through the cuvette and returned to the animal via a catheter in the jugular vein. When the cuvette was in use the pressure transducer was connected to a side arm of the carotid cannula. In some experiments a carotid artery–carotid artery loop was used rather than carotid artery-jugular vein loop. Although this gave satisfactory measurements of P_{a,O_2} at normal heart rates, at low blood flow rates, as occurred during diving, the time necessary to flush the cannula to the oxygen electrode was too long for any confidence to be placed in data for P_{a,O_2}. This problem was not encountered with the arterio-venous loop. The 90 % response time of the electrode was set by the nature of the membrane covering the electrode and the electronics and was usually less than 1·7 sec. The electrode was calibrated using air or N_2 equilibrated saline, at 37 °C, applied to the side arm of a T-piece on the upstream end of the cannula and removed through a T-piece on the downstream side. Calibration salines were flowed past the electrode from a pressure head which was adjusted to be close to that of the animal's blood pressure. The cuvette containing the electrode was enclosed in a water jacket which was maintained at 37 °C by a continuous flow of heated water.

(*b*) *Artificial ventilation and measurement of spontaneous respiration.* Breathing was monitored by a miniature pneumotachograph attached to a tracheal cannula, by a thermistor inserted through the upper jaw into the internal nares, or by measuring the impedance change across the chest between the tips of two subcutaneous wires attached to a Harvard impedance pneumotachograph. The tracheal cannula was inserted in the middle neck region after exposing the trachea as described above. The flow signal obtained from the pneumotachograph was integrated to give tidal volume. Artificial ventilation was performed using a small positive pressure pump. The pump contained a rotary valve, the revolutions of which set ventilation frequency while the proportionate length of the inspiratory to expiratory period could be altered by manually selecting the position of the inspiratory and expiratory tubes of the pump with respect to the rotary valve (Drummond, 1979). Inflation pressure was set by a water column on the gas line connecting the pump to the gas supply. Maintained lung inflation (to test for the Hering–Breuer

reflex) was achieved by clamping the tube carrying expired gas. Lung collapse in paralysed animals was achieved by clamping the inspiratory tubing. In a large number of experiments another cannula was inserted into the trachea pointing towards the mouth (oral facing tracheal cannula) and was used for passing water across the internal respiratory passages.

(*c*) *Measurement of heart rate.* Heart rate was obtained from the e.c.g., the latter being recorded with bipolar copper wire electrodes. One electrode was placed subcutaneously over the heart and the other subcutaneously in the left hind limb. To obtain heart rate in voluntary dives the electrode wires were passed subcutaneously to a miniature connector which was anchored between the clavicles. The skin was closed, as much as possible, around the base of the miniature connector and the animal allowed to recover overnight before experimentation began.

(*d*) *Nerve section procedures.* The olfactory nerve, facial and superior petrosal nerves, mandibular, opthalmic and maxillary branches of the trigeminal nerve were exposed intracranially after decerebration. The inferior and superior laryngeal, glossopharyngeal, sinus, vagus and phrenic nerves were approached ventrally through an incision running from the lower jaw to the thorax. Nerves were either sectioned or cooled to 2–3 °C by placing them on a silver block through which cooled acetone was circulated. Cooling was as effective as section for eliminating activity in a given nerve. Bipolar silver wire hook electrodes were used to record either sensory or motor activity from the cut ends of nerves. Similar electrodes were used to stimulate the central ends of the trigeminal branches and inferior and superior laryngeal nerves. The electrodes were connected to a Grass S-6 stimulator (Grass Instruments Inc., Quincy, Mass.) through a stimulus isolation unit. Lung deafferentation was effected by forced inflation of the lungs with steam (Hainsworth, Jacobs & Comroe, 1973). A T-piece was attached to the end of the tracheal cannula and a heavily insulated tube, with a side arm close to the trachea, was run from a 2·0 l. conical flask to a stopcock on one side of the T-piece. Water in the flask was brought to a rolling boil and the steam exited through the side arm on the insulated tube. The side arm and free side of the tracheal T-piece were closed and, at the same time, the stopcock on the T-piece opened so that the animal received one lung inflation with steam. This procedure was repeated, after 2–3 min, until the animal had received up to six individual inflations with steam.

(*e*) *Signal recording and analysis.* All signals were amplified by conventional means and blood pressure, P_{a,O_2} breathing frequency or integrated tracheal air flow, the e.c.g., e.n.g.s. or pulse frequency, were displayed on both a Tektronix storage oscilloscope and either a Harvard four-channel or Beckman two-channel pen writer, both writing on curvilinear co-ordinates. E.n.g.s, along with an event trace, were also recorded on a Tanberg 2-channel tape system for later analysis using a window discriminator and ratemeter.

(2) *Experimental protocol*

(*a*) *Diving under 'natural' and laboratory conditions.* Five muskrats, instrumented to give e.c.g., were placed in a holding tank, 4 m diameter and 1·5 m deep filled with water to a depth of 1 m. A platform (0·5 m × 0·5 m square) was floated on the water surface and the animals could move freely from water to 'land'. A long thin insulated wire was connected from the animal to the recording apparatus. The signal was amplified conventionally and displayed on a 2-channel pen recorder along with an event marker which was activated to indicate submergence of the animal. One of us observed the animals at all times and when they submerged, pressed the event marker ('free range' dive). These animals were then taken into the laboratory and submerged to monitor e.c.g. during restrained diving. The animals were placed in a sieve-like Perspex box and restrained by placing metal bars across the box around the outline of the muskrat. A box full of water was raised from beneath the animal box to completely submerge the muskrat. In these experiments the dive time was at most 40 sec, which was equal to the longest dive time recorded for animals in 'free range' dives. A further group of animals was anaesthetized with urethane and submerged as described for restrained diving of unanaesthetized animals.

(*b*) *Stimulation of external nares and internal respiratory passages.* Nasal stimulation was effected by passing water or saline through the oral facing tracheal cannula with the animal on its back. A flow of 32 ml. min^{-1} was generated when the reservoir was held 1 m above the animal. Reducing the pressure head proportionately reduced the fluid flow rate. At the end of a period of nasal stimulation air was blown through the oral facing cannula to remove all the fluid from

the internal nasal passages. Just before another bout of nasal stimulation the water was run to the end of the cannula which was then clamped. Removing the clamp applied water to the nasal area with a lag time of less than one second. Water stimulation of the external narial region alone was done in four curarized artificially ventilated muskrats. The animal was supine and the nose was pushed through a small hole in a piece of dental dam which formed one side wall of a watertight box. Air was continuously blown through the oral facing cannula and this exited through the nares preventing the water from entering. The water level in the box was raised by pouring water from a beaker into the open top or by raising the water level from a reservoir and it was drained by removing a plug from the bottom of the box. The effect of decerebration and of sectioning the cranial nerves mentioned previously on the respiratory and cardiovascular responses to nasal stimulation were studied. The delimitation of the neural pathway for the responses was confirmed by recording from receptors innervated by the cranial nerves and by stimulating the central cut ends of these nerves to mimic afferent activity.

(c) *Effects of input from pulmonary receptors.* The effect of lung receptor input on the responses to nasal stimulation was studied in animals which were artificially ventilated over a range of pressures from 0·4 to 2·0 kPa. The ventilation was maintained throughout periods of nasal stimulation with water and saline, before and after lung deafferentation. The effect of maintained inflation or deflation of the lungs on heart rate, blood pressure, and P_{a,O_2} was investigated in paralysed animals.

(d) *Bilateral section of the carotid sinus nerves.* Baroreceptors were activated by the increase in blood pressure following intra-arterial injection of adrenaline 5 μg/kg, while carotid body chemoreceptor stimulation was achieved by injection of 80–200 μg/kg KCN into the carotid artery. Baroreceptor denervation was achieved by section of the sinus nerve (IX) and was judged to be successful when the blood pressure rise following adrenaline injection failed to cause bradycardia. The carotid body chemoreceptors were also denervated by sinus nerve section which was confirmed by the fact that there was no increase in breathing following KCN injection. The effects of combined carotid baroreceptor and chemoreceptor denervation on the responses to nasal stimulation and maintained lung inflation and deflation were investigated. In some experiments chemoreceptors were stimulated by artificially ventilating the animal, at control frequency and tidal volume, with a gas mixture of 5% CO_2 in nitrogen. P_{a,O_2} was continuously monitored during this procedure. The cardiovascular responses to breathing 5% CO_2 in N_2 were recorded before and after bilateral section of the carotid sinus nerves. Heart rate and P_{a,O_2} were also monitored continuously during nasal stimulation, with and without maintained artificial ventilation, and during asphyxia.

RESULTS

1. *Cardiovascular and respiratory responses to diving under 'natural' and laboratory conditions*

Heart rate of five animals held in large outdoor tanks averaged 310 ± 3 min^{-1} ($n = 102$) and fell to 54 ± 3 min^{-1} ($n = 102$) 1–2 sec after voluntary submergence. Heart rate continued to decline, as submergence was prolonged, and fell to 27 ± 3 min^{-1} ($n = 3$) at 40 sec. Breathing started immediately on emergence and heart rate increased to the pre-dive rate within 5 sec. In one animal the onset of the cardiac response preceded submergence while in all animals it appeared that bradycardia was related to the length of the dive; in fifty dives of less than 5 sec, selected at random from all data, the first cardiac interval after submergence was the longest, lasting $0{\cdot}82 \pm 0{\cdot}03$ sec, whereas in longer dives the first cardiac interval nearly doubled to $1{\cdot}56 \pm 0{\cdot}08$ sec ($n = 37$) and the second or third intervals were usually longer. In no case was evidence obtained for an anticipatory increase in heart rate before emergence.

Mean heart rate of ten restrained muskrats in the laboratory (266 ± 3 min^{-1}; $n = 66$) was significantly lower than in unrestrained animals. Nostril closure and

apnoea occurred on submergence and prominent bradycardia after a delay of 300 ± 10 msec ($n = 60$), heart rate falling to 78 ± 4 min^{-1} ($n = 60$). A stable rate of 51 ± 2 min^{-1} ($n = 60$) was reached after 5 sec submergence and was maintained until surfacing after 40 sec. On emergence heart rate increased markedly with the first breath and the pre-dive rate was reached after 10 sec; there was no post-dive tachycardia.

Submerging four surgically anaesthetized muskrats (urethane, I.P.) caused similar changes in heart rate as was seen in unanaesthetized restrained muskrats. Heart rate fell from 281 ± 5 to 84 ± 4 min^{-1} ($n = 56$) after 591 ± 40 msec and continued to decline to 39 ± 2 min^{-1} ($n = 39$) after 40 sec under water. Generally, the anaesthetized animals continued to make some breathing movements during submergence. Confirmation that water was drawn into the internal nares and then expelled was obtained by locating a bead thermistor in the internal nasal passages and noting the temperature changes accompanying this activity. On emergence, breathing movements were sporadic and heart rate rose slowly, sinus arrhythmia being prominent. It was unusual for heart rate to reach pre-dive levels in the first minute after surfacing. Diving bradycardia was relatively unaffected by decerebration at the thalamic level (ten animals) but was abolished by bilateral vagotomy (three animals).

2. *Apnoea and bradycardia caused by stimulation of the external nares and internal respiratory passages*

Pouring water over the external nares of four curarized muskrats, artificially ventilated at resting minute volumes, caused bradycardia. Heart rate fell on average from control values of 292 ± 6 ($n = 6$) to 76 ± 12 min^{-1} ($n = 6$) 1 sec after pouring the water. Bradycardia was maintained throughout the period that water covered the nose (30–60 sec). Local anaesthesia of the narial region or covering the external nares with petroleum jelly eliminated the maintained bradycardia in response to water on the nose although a transient period of bradycardia (3–5 sec) was often seen on complete submergence of the nose. A transient period of bradycardia was also evoked by manually stroking the fur.

In the majority of these trials water was prevented from entering the internal nares by maintaining a continuous air flow, out through the nares, from an oral facing tracheal cannula. However, allowing water to enter the internal nares caused a sustained and more pronounced bradycardia. Passing water or saline through the oral facing cannula, and out of the nares, in anaesthetized or curarized muskrats caused varying degrees of apnoea and bradycardia depending on the water flow rate or ionic composition of the fluid.

Nasal water flow of 32 ml. min^{-1} caused apnoea and heart rate to fall from 277 ± 5 ($n = 34$, twelve animals) to 20 ± 2 min^{-1}, within one third of a second from the first appearance of water in the trachea. However, heart rate increased to reach 33 ± 2 min^{-1} after 20 sec of water flow. Mean arterial blood pressure fell with the bradycardia but increased and was usually above control levels when heart rate increased after 10–20 sec of water flow. After 20–40 sec of nasal water flow the animals generally began to make small respiratory efforts which increased in depth and frequency with time. Heart rate rose but full recovery of breathing and heart rate did not occur

until well after flow was stopped and water cleared from the nasal region. Repeating water flow periods, with only a short time (60 sec) between them, caused a diminution in both the sustained period of apnoea and bradycardia. In fact, ten periods of water flow (20–30 sec duration) in quick succession were usually sufficient virtually to eliminate both the apnoeic and bradycardic responses. Therefore, to obtain standard results we always allowed at least 15 min between each water flow period and evacuated the nasal passages between flow periods.

Reducing the water flow rate (10–15 ml. min^{-1}) or substituting saline (0·9 % NaCl) for water at higher flow rates diminished the initial apnoeic period as well as the depth of bradycardia, particularly during prolonged flow periods (about 40 sec). In the initial period of saline flow, when animals were apnoeic, heart rate (38 ± 3 min^{-1}, $n = 12$) was significantly above that from the same animals with nasal water flow of 32 ml. min^{-1}. Within 10 sec from the start of flow an inspiration was made which was invariably preceded by a heart beat. Both tidal volume and breathing rate increased throughout the period of stimulation and so therefore did heart rate, especially since after 20 sec or so of flow two heart beats occurred with each breath, one preceding and one coinciding with peak inspiration. Consequently, in these trials maintained

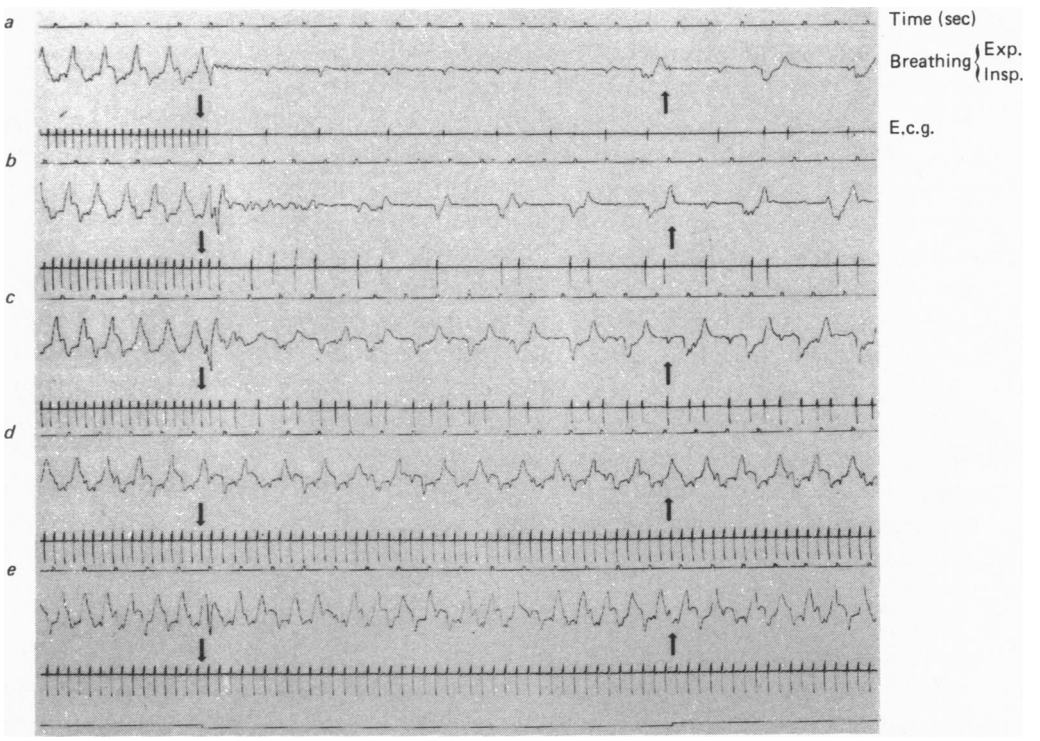

Fig. 1. The effect of unilateral and bilateral section of the inferior laryngeal (X) and maxillary branch of the trigeminal nerves (V) on the cardiovascular and respiratory response to nasal water flow (32 ml. min^{-1}) in a decerebrated muskrat. Traces from top to bottom, time (1 sec); tracheal air flow (inspiration downward); e.c.g. *a*, response before nerve section; *b*, after left X section; *c*, bilateral X section; *d*, after bilateral X and left V section; *e*, after bilateral X and V section. Nasal water flow started at the downward pointing arrow and stopped at the upward pointing arrow.

bradycardia was directly dependent on the stimulus effectiveness in causing apnoea.

The afferent pathway of the water flow response was investigated by means of nerve section, reversible nerve cooling or stimulation of the central cut ends of sectioned nerves in fourteen animals of which eleven were decerebrate. Decerebration had little effect on the apnoeic response to nasal water flow but the bradycardia was not as great compared with anaesthetized controls. Decerebration had no effect on resting heart rate, breathing frequency or tidal volume (Fig. 1, Table 1). Bilateral section or cooling blockade of the following nerves had no effect on either the respiratory or cardiac responses to water flow; I olfactory ($n = 10$), V ophthalmic ($n = 10$), V mandibular ($n = 10$), VII main ($n = 2$), VII superior petrosal ($n = 3$), IX main, distal to sinus nerve ($n = 3$), IX sinus ($n = 5$), X superior laryngeal ($n = 6$), X recurrent laryngeal ($n = 3$). However, unilateral nerve stimulation (1 V, 10 Hz, 10 msec duration) of the nasociliary branch of opthalmic V gave transient bradycardia and apnoea in two of five animals and prompt transient responses were always elicited by stimulation of the sinus nerve. Bilateral section or reversible cold block of the maxillary branch of V and the inferior laryngeal (X) completely eliminated both the cardiac and respiratory response to water flow (Fig. 1), although bilateral section of maxillary V alone had more effect on the cardiac response than bilaterally sectioning the inferior laryngeal (X) alone (Table 1). Unilateral electrical stimulation (1 V, 10 Hz, 10 msec) of maxillary V or the inferior laryngeal (X) caused sustained apnoea and bradycardia. Electroneurograms taken from a small slip of the inferior laryngeal (X) in three muskrats displayed a continuous discharge between 30 and 45 impulses sec^{-1}. Water flow caused discharge to increase abruptly, some 4–5 times, and the elevated discharge rate was often maintained for a minute or more after cessation of flow. Letting a 0·5 % solution of Xylocaine stand in the internal nasal passages abolished the control discharge and there was no effect of water flow on discharge. Further, the respiratory and cardiac responses to water flow were lost.

In two anaesthetized decerebrate muskrats the pharynx and palate were exposed by a mid-ventral section of the lower jaw and the internal respiratory passages probed with a blunt rod. The dorsal anterior surface of the soft palate and a pharyngeal region rostral to the glottis were the most sensitive to punctuate stimulation as judged from the cardiac and respiratory responses evoked. The former region was innervated by the nasopalatine branch of maxillary V and the latter by the inferior laryngeal (X).

3. *Effect of artificial ventilation on bradycardia caused by nasal stimulation and bradycardia associated with apnoea alone*

Artificial ventilation of seventeen anaesthetized muskrats in air caused slight cardio-acceleration when minute volume exceeded that during unassisted ventilation. At four times the normal minute volume of 150 ± 4 ml. min^{-1} ($n = 10$) heart rate increased by 10 beats min^{-1}. This effect was independent of CO_2 washout as it occurred in animals ventilated with 95 % O_2 and 5 % CO_2.

It was evident that artificial ventilation drove respiratory motor neurone output, monitored by phrenic nerve discharge, when the rate was close to the normal breathing rate (70 ± 6 min^{-1}, $n = 10$) and tidal volume was above the resting tidal volume

TABLE 1. The effect of nasal water flow (32 ml. min^{-1}) on heart frequency and minute volume in fourteen muskrats before and after bilateral section or reversible cold block of inferior laryngeal (X) and maxillary (V) nerve. Each water flow period was 30 sec and heart rates and minute volumes are the averages for this period. On average each test was done 3 times on each animal and N = number of animals used

	Normal animals		Thalamic decerebrate		Water flow		Water flow	
	Control	Water flow	Control	Water flow	After bilateral inferior laryngeal (X) section alone	After bilateral X and maxillary V section	After bilateral maxillary V section alone	After bilateral V and inferior laryngeal (X) section
Heart rate beats.min^{-1}	277 ± 13	25 ± 4	286 ± 13	53 ± 10	93 ± 18	270 ± 18	177 ± 14	275 ± 8
Minute volume ml. min^{-1}	214 ± 8	0	189 ± 12	22 ± 10	101 ± 15	171 ± 9	89 ± 18	181 ± 17
N	14	14	11	11	7	3	7	3

of $2{\cdot}3 \pm 0{\cdot}2$ ml. ($n = 10$). Maintaining this level of ventilation throughout a period of nasal water or saline flow always affected the level of bradycardia, although no level of ventilation was found which completely eliminated a short period of bradycardia at the start of nasal stimulation (Figs. 2 and 3). The later development of bradycardia depended on the quality of the nasal stimulation. With low water flow rates (14 ml. min^{-1}) or saline flows (weak nasal stimulation) there was a tendency for bradycardia to be progressively overruled as tidal volume was increased above the

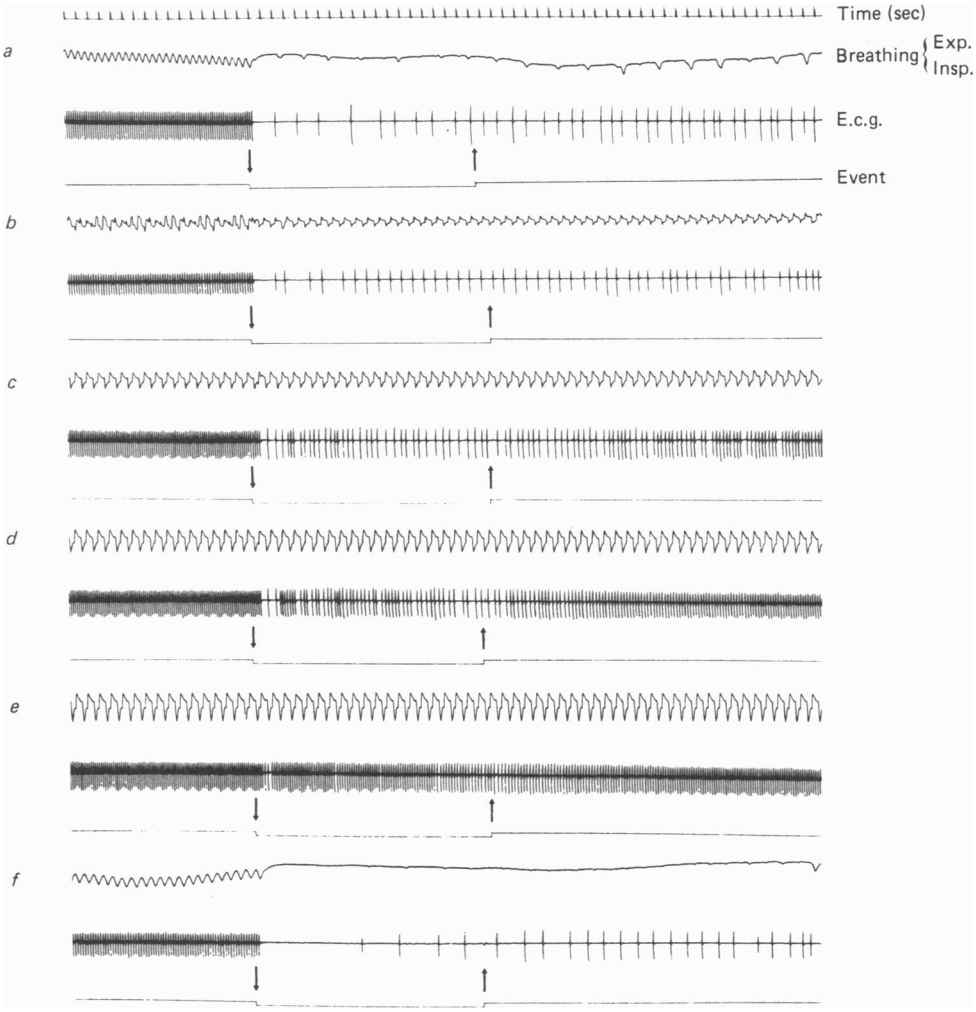

Fig. 2. The effect of artificial ventilation, at progressively increased inspiratory pressures, on the cardiovascular response to flow of saline through the nasal passages. In each set of traces the top is time (1 sec), next, tracheal air flow (inspiration downward), next, e.c.g. and the bottom is an event marker (down on trace = nasal flow started, up on trace = nasal flow stopped, this trace is accentuated by the downward and upward pointing arrows); *a*, effect of saline flow in a spontaneously breathing animal; *b* to *e*, forced ventilation at 0·4, 0·8, 1·2 and 1·6 kPa inspiratory pressures respectively; *f*, effect of water flow (32 ml. min^{-1}) in the spontaneously breathing animal.

normal resting value (Fig. 2). The relationship between minute volume and heart rate during low water flow rates or saline flows in non-ventilated and artificially ventilated animals showed that the heart rate obtained at any given level of spontaneous ventilation was only achieved, in artificially ventilated animals, when minute volume was increased 4–5 times. On the other hand, with high nasal water flows (strong nasal stimulation) (32 ml. min^{-1}), artificial ventilation was without apparent effect on bradycardia at low tidal volumes. With tidal volume above

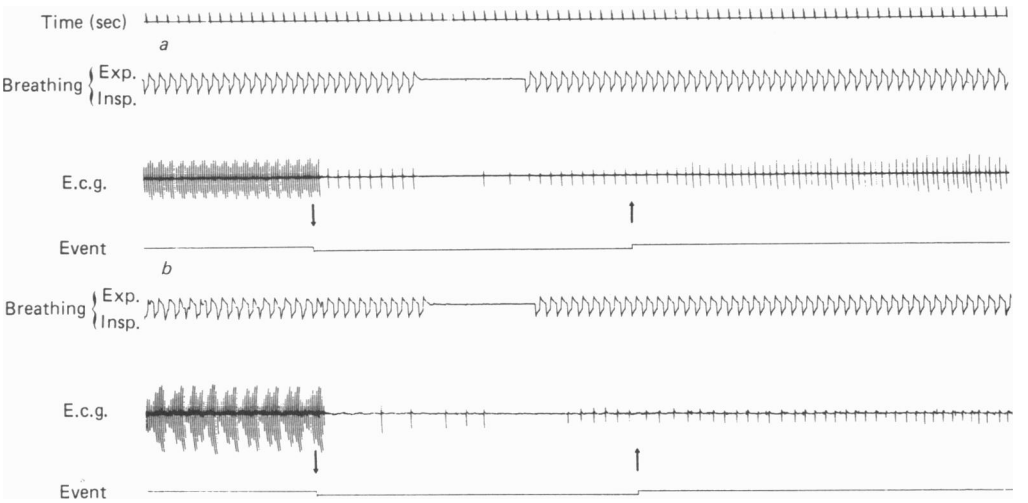

Fig. 3. The effect of maintained artificial ventilation on the cardiac response to nasal water flow (between arrows; 32 ml. min^{-1}) before (*a*) and after lung deafferentation (*b*). Top trace in Figure, time (1 sec). Traces in *a* and *b* from top to bottom, tracheal air flow (inspiration downwards, inspiratory pressure 1·2 kPa), e.c.g. and event trace (down = water flow on, up = water off, accentuated by arrows). The respiratory pump was switched off during nasal water flow to show more clearly the effects of artificial ventilation on heart rate in the intact animal.

4 ml., heart rate was 'locked' to respiratory frequency in the range of normal respiratory frequencies (Fig. 3). Substituting 95 % O_2 and 5 % CO_2 for air during artificial ventilation had no effect on the relationship between cardiac and ventilation frequencies during nasal stimulation. Slowing the ventilation rates during stimulation caused the heart to beat irregularly, but at a faster rate than in non-ventilated preparations, while at high ventilation rates and volumes (8 ml.) it was not unusual for heart rate to remain high during the initial period of flow, falling to ventilation frequency after a variable period of 5–10 sec.

The effect of maintained lung inflation on the bradycardia caused by nasal water flow was investigated in trials on three muskrats by comparing heart rate during maintained inflation with that in free breathing muskrats (in which the lungs collapsed at the onset of flow since the tracheal cannula bypassed the glottis). Maintained inflation at a pressure of 1·0–1·5 kPa caused a period of 15–20 sec of apnoea. Applying a strong nasal stimulus at the start of the inflation period gave the same level of bradycardia as was observed in free breathing animals.

Deafferentation of the lungs was achieved by allowing the animals to breath steam. Two breaths of steam abolished the Hering–Breuer inflation reflex yet heart rate was still governed by artificial ventilation frequency during strong nasal stimulation. After four breaths of steam continuous artificial ventilation at 1 kPa had no apparent effect on the bradycardia caused by nasal stimulation (Fig. 3).

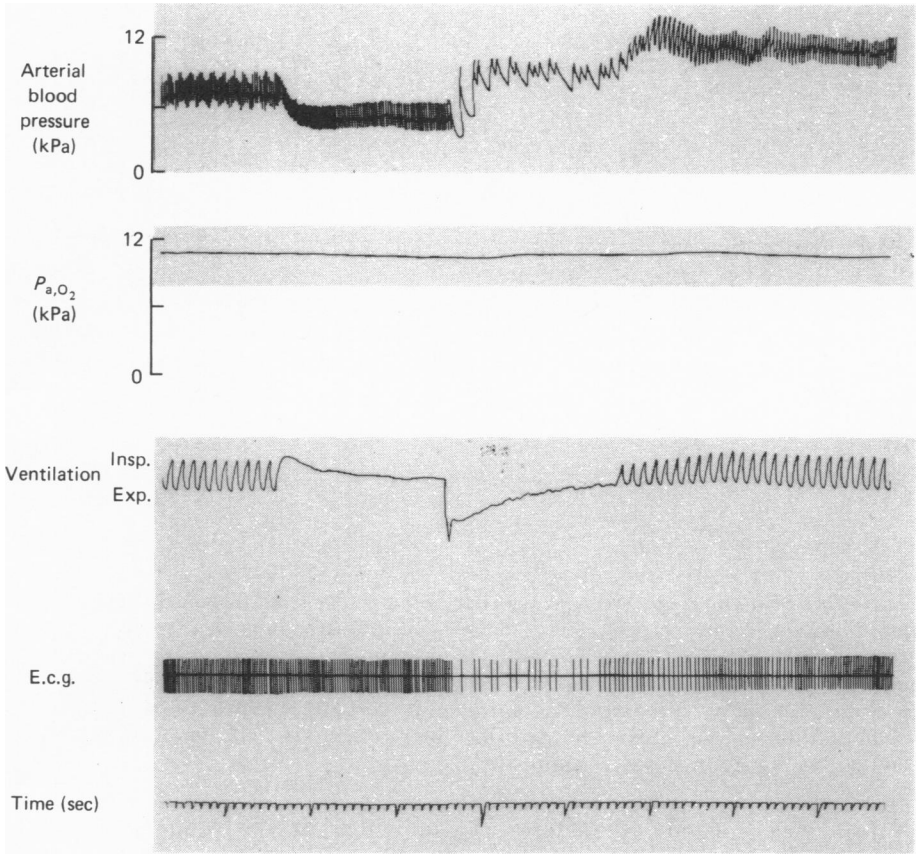

Fig. 4. The effect of maintained lung inflation and deflation on the heart rate of a paralysed muskrat. Traces from top to bottom, arterial blood pressure (kPa), arterial O_2 tension recorded continuously (kPa), ventilation monitored as an impedance pneumogram (up on trace = inspiration, inflation pressure = 1·2 kPa), e.c.g., time (1 sec). Lungs inflated at a pressure of 1·2 kPa.

In free breathing animals complete lung deafferentation had little effect on minute volume (before $150 \pm 4{\cdot}0$ ml. min^{-1}, $n = 10$; after 161 ± 24 ml. min^{-1}, $n = 10$) since, although tidal volume increased by 3 times (before $2{\cdot}3 \pm 0{\cdot}2$ ml., $n = 10$; after $6{\cdot}7 \pm 0{\cdot}3$ ml., $n = 10$), respiratory frequency fell to one third the normal rate (before 70 ± 6 min^{-1}, $n = 10$; after 24 ± 2 min^{-1}, $n = 10$). Heart rate in free breathing controls was $286 \pm 5{\cdot}9$ ($n = 10$) and this fell to $195 \pm 21{\cdot}7$ ($n = 10$) in lung denervates. Sinus arrythmia was prominent in about half the deafferentated animals. Strong nasal stimulation caused heart rate to fall to $21{\cdot}3 \pm 3$ min^{-1} ($n = 10$) in intact free

breathing muskrats yet in denervates heart rate only fell to $48{\cdot}5 \pm 2$ min^{-1} ($n = 9$). There was no apparent effect of deafferentation on the respiratory response to nasal water flow.

Whether lung deflation *per se* had any effect on cardiac frequency in the absence of nasal stimulation was investigated in nine artificially ventilated curarized muskrats. The animals were artificially ventilated with O_2 and P_{a,O_2}, measured continuously, remained above 10·3 kPa throughout all tests. Stopping artificial ventilation with lungs deflated caused bradycardia within $0{\cdot}97 \pm 0{\cdot}17$ sec ($n = 28$) (Fig. 4). Heart rate fell from 268 ± 7 to 59 ± 4 min^{-1} ($n = 23$) and neither the latent period nor level of bradycardia was affected by the pump pressure over a range 0·4–1·6 kPa. Blood pressure rose sharply with onset of bradycardia and stabilized within 2–4 sec considerably above control values (Fig. 4). Denervation of arterial baroreceptors by sinus nerve section had no effect on the hypertensive response to lung deflation. Clamping the expiratory hose during inspiration (maintained lung inflation) also caused bradycardia but the onset was delayed $6{\cdot}8 \pm 1{\cdot}8$ sec ($n = 5$) at an inflation pressure of 0·5 kPa to 20 ± 3 sec ($n = 17$) at a pressure of 1·0 kPa to 35 ± 7 sec ($n = 8$) at a pressure of 1·5 kPa. Although maintained inflation was accompanied by prominent hypotension (Fig. 4), denervation of arterial baroreceptors had no effect on either the cardiac or blood pressure response to inflation.

4. Bradycardia caused by stimulation of arterial baroreceptors and carotid body chemoreceptors

Injection of 5 μg.kg^{-1} adrenaline caused a marked elevation in blood pressure from $8{\cdot}6 \pm 0{\cdot}66$ ($n = 6$) to $16 \pm 0{\cdot}66$ kPa. On average, bradycardia appeared when mean blood pressure reached $15{\cdot}7 \pm 0{\cdot}5$ kPa ($n = 6$), heart rate falling from 253 ± 6 to 90 ± 12 beats.min^{-1} in spite of any stimulatory effect of adrenaline *per se* on the heart. Injection of 80–200 μg.kg^{-1} cyanide into the carotid artery caused hyperpnoea and tachycardia in spontaneously breathing muskrats and transient bradycardia in artificially ventilated muskrats. Section of the carotid sinus nerves eliminated both respiratory and cardiac responses to injection of cyanide and the bradycardia associated with adrenaline induced hypertension.

Sinus nerve section had no effect on the bradycardia caused by nasal stimulation or lung deflation despite the fact that, in intact animals, hypertension was associated with bradycardia. On the other hand the P_{a,O_2} at which bradycardia occurred, in tests in which animals were ventilated with 5% CO_2 in N_2, was considerably reduced. Artificial ventilation of curarized muskrats with 5% CO_2 in N_2 caused bradycardia when P_{a,O_2} reached $8{\cdot}4 \pm 0{\cdot}8$ kPa ($n = 6$). Heart rate fell from the initial rate of 277 ± 11 to 76 ± 7 beats min^{-1} at a P_{a,O_2} of 4 kPa (Fig. 5). Mean arterial pressure was little affected in the early period of ventilation with 5% CO_2 in N_2 but rose when bradycardia developed (Fig. 5). Bilateral section of the sinus nerves delayed bradycardia in similar tests until P_{a,O_2} fell to $4{\cdot}5 \pm 0{\cdot}5$ kPa ($n = 9$) (Fig. 6).

To assess further the contribution of chemoreceptors to diving, P_{a,O_2} was continuously monitored in paralysed muskrats during tracheal clamping, with and without simultaneous nasal water flow, and when artificial ventilation was maintained during nasal water flow. In the latter condition heart rate fell from 282 ± 16 ($n = 12$) to 81 ± 10 min^{-1} ($n = 12$) during flow and P_{a,O_2} increased by some 5%. During

tracheal clamping, with or without simultaneous nasal water flow, there was a latent period before P_{a,O_2} declined. In the case of tracheal clamping alone the latent period was $7{\cdot}3 \pm 1{\cdot}2$ sec and decline in P_{a,O_2} accompanied development of bradycardia.

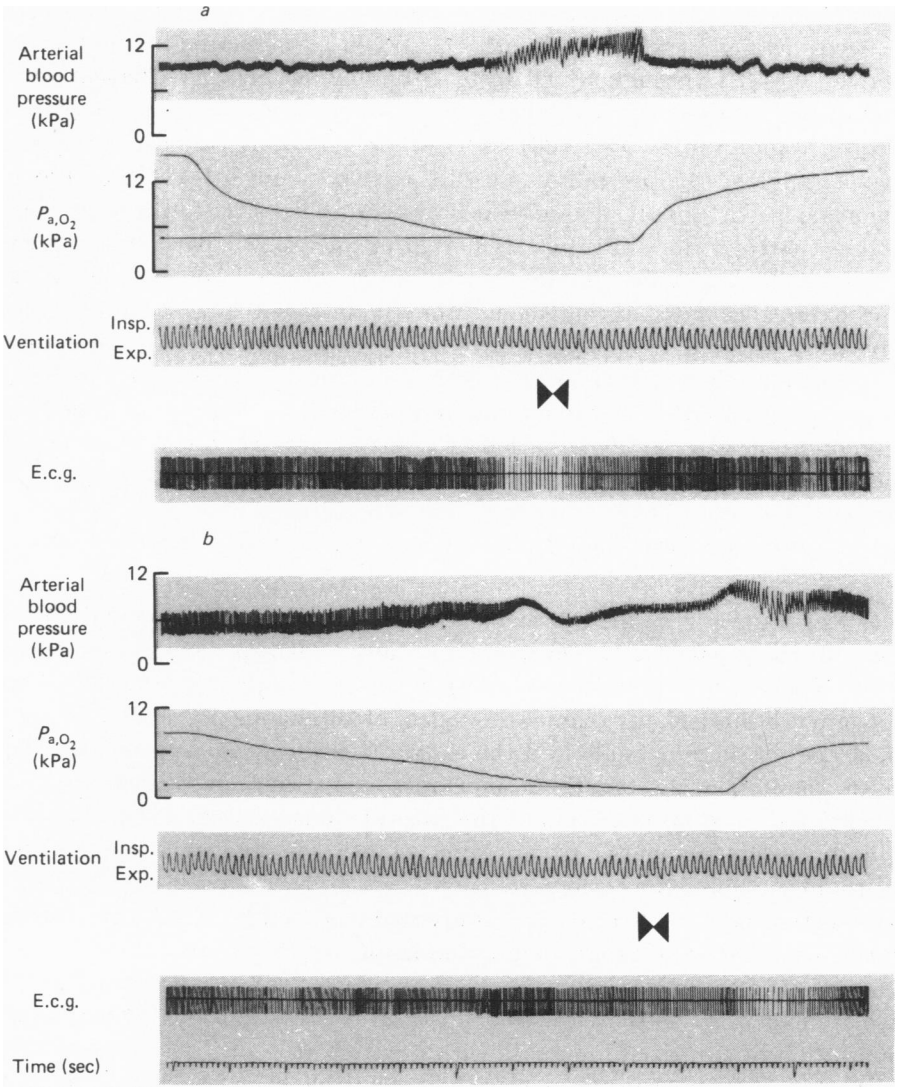

Fig. 5. The effect of sinus nerve section on the bradycardia resulting from ventilating a paralysed muskrat with 95 % N_2 and 5 % CO_2; *a*, intact; *b*, after bilateral sinus nerve section. In *a* and *b*, traces from top to bottom are, arterial blood pressure (kPa), arterial oxygen tension (kPa), impedance pneumogram showing ventilation (up on trace = inflation), e.c.g. The bottom trace in the Figure is a 1 sec time marker and applies to both *a* and *b*. Bradycardia was recognized when cardiac interval lengthened by 10 % and the horizontal arrow marks the P_{a,O_2} at which this occurred. The opposed arrow heads mark when the ventilating gas was changed from 95 % N_2 and 5 % CO_2 to 100 % O_2.

Water flow and tracheal clamping caused rapid initiation of bradycardia and the latent period before any change in P_{a,O_2} was extended to $11{\cdot}4 \pm 1{\cdot}5$ sec. P_{a,O_2} fell more rapidly in the case of tracheal clamping alone and after 60 sec was $15 \pm 1{\cdot}5\,\%$ of initial P_{a,O_2} (initial $P_{a,O_2} = 12{\cdot}9 \pm 0{\cdot}34$ kPa) whereas tracheal clamping plus nasal stimulation caused a more rapid development of bradycardia and, consequently, a slower utilization of the blood oxygen store for, at 60 sec, P_{a,O_2}, had only fallen to $25 \pm 3{\cdot}0\,\%$ of initial P_{a,O_2} (initial $P_{a,O_2} = 12 \pm 0{\cdot}4$ kPa).

DISCUSSION

Diving bradycardia in the muskrat is similar in voluntary unrestrained dives to that observed in forced submergence of restrained animals. In this respect, muskrats resemble other diving mammals (Elsner, 1965; Elsner, Franklin, Van Citters & Kenney, 1966; Kooyman & Campbell, 1972; Jones *et al.*, 1973) and are quite unlike birds which display a profound alteration of the 'laboratory-type' diving response in the wild (Butler & Woakes, 1975, 1976). However, two features of unrestrained diving were never seen in laboratory dives. In one animal, heart rate was reduced before submergence in voluntary dives indicating a measure of conditioning of the cardiovascular response. Furthermore, the bradycardia established on submergence was roughly proportional to the length of the dive which ensued. This has also been observed in seals making voluntary dives (Kooyman & Campbell, 1972).

Diving bradycardia persisted under anaesthesia although the apnoeic response was weaker and anaesthetized muskrats took water into the internal narial passages but it did not pass the glottis. The maintenance of the cardiovascular response during anaesthesia did allow us however to differentiate between the cardio-depressor effects of various receptor groups.

Stimulation of the external nares with water, during maintained artificial ventilation in paralysed animals, caused bradycardia which was virtually eliminated by covering the area with vaseline or injecting the external nares with local anaesthetic. Passing water from the trachea through the internal respiratory passages caused more pronounced bradycardia and apnoea in the expiratory phase. Both the apnoeic and cardiac responses were significantly reduced by substituting saline for water in these trials. Consequently, the potential for causing apnoea and bradycardia exists in several groupings of receptors on both external and internal narial regions in the muskrat. Angell-James & Daly (1969, 1972) have reviewed the subject of nasal reflexes of this type in mammals and suggest that the receptors involved are free nerve endings, which are innervated by the trigeminal nerve. Bilateral section of the trigeminal nerves, however, failed to eliminate completely the respiratory and cardiac responses to nasal water flow in muskrats. Only bilateral section of the inferior laryngeal nerves, along with bilateral trigeminal section, completely eliminated respiratory and cardiac responses to nasal stimulation. The inferior and superior laryngeal nerves had been previously identified as the afferent pathway for the diving reflex in sheep also (Tchobroutsky, Merlet & Rey, 1969).

Maintained lung inflation, in the absence of nasal stimulation, caused marked hypotension with, initially, little effect on heart rate. In the dog, lung stretch receptors are responsible for initiating a vasomotor reflex causing vasodilatation in

skin, muscle and splanchnic vascular beds during maintained lung inflation (Daly *et al.* 1967; Daly & Robinson, 1968) and they may contribute to the hypotension seen in muskrats. As lung inflation is maintained bradycardia occurs, the latent period to bradycardia being directly proportional to the inflation pressure in the absence of any change in P_{a,O_2}. Bradycardia in this case must be caused by changes in pulmonary input either due to receptor adaptation or the prolonged absence of dynamic changes. A relationship between heart rate and respiratory motor neurone discharge was confirmed by the sinus arrhythmia often displayed by lung denervates. A sudden lung deflation in intact animals caused a prominent bradycardia similar in magnitude to that observed during unrestrained dives. This response does not depend on the presence of an intact barostatic reflex, unlike that seen in the rabbit (White & McRitchie, 1973), and is independent of changes in P_{a,O_2} or the inflation pressure attained during the previous ventilation.

Artificial ventilation decreased the cardiac response to nasal stimulation. In the case of saline stimulation of the internal respiratory passages the bradycardic response may be almost completely overruled at minute volumes some 3–4 times normal resting minute volumes. Large tidal volumes at a rate close to the normal breathing rate pace the central respiratory neurones. It appears that if nasal stimulation is relatively weak, particularly in anaesthetized muskrats, then continued central and peripheral respiratory related activities during stimulation prevent bradycardia. Paradoxically, strong nasal stimulation (high water flow rate) often yields a 1:1 relation between artificial ventilation frequency and heart rate when tidal volume is increased above normal. Strong nasal stimulation may completely inhibit central respiratory neurone discharge so that only peripheral input now affects heart rate. Certainly after lung deafferentation 'pacing' of heart beat by ventilation was not seen. In mammals and birds it is well established that lung inflation will reverse bradycardia (Anrep *et al.* 1936*a*, *b*; Daly & Scott, 1958: Bamford & Jones, 1976; Angell-James & Daly, 1978) the response being due to input from lung receptors rather than changes in blood gas tensions (Bamford & Jones, 1976; Angell-James & Daly, 1978). However, in muskrats it appears that the static, pressure related, pulmonary afferent information causing inflation apnoea (Hering–Breuer reflex), is not responsible for the cardiac pacing seen during strong nasal stimulation. Inflation apnoea disappears after one to two breaths of steam but cardiac pacing persists. Furthermore, the response to nasal water flow is not inhibited by maintained inflation of the lungs during nasal stimulation. Receptors within the lungs of mammals respond primarily to one or both of the rate of change and magnitude of transpulmonary pressure (Adrian, 1933; Davis, Fowler & Lambert, 1956). In muskrats the rate sensitive discharge of pulmonary receptors seems to drive heart rate.

During artificial ventilation with 5% CO_2 in N_2 bradycardia occurred when P_{a,O_2} reached 8 kPa. Bilateral section of the carotid sinus nerves reduced the cardiovascular sensitivity to change in P_{a,O_2} for now bradycardia was delayed until P_{a,O_2} reached 4 kPa. There is some doubt about the role of aortic bodies in affecting heart rate (Daly, 1972) so it is pointless to speculate about the agents causing the bradycardia after carotid sinus denervation. In prolonged dives P_{a,O_2} will decline to 8 kPa within 20 sec so it is reasonable to expect the carotid body chemoreceptors to be stimulated

and contribute to bradycardia at this time. However, the chemoreceptor driven bradycardia was investigated during artificial ventilation which, as already discussed, will have a stimulatory effect on heart rate. Therefore, it is possible that the chemoreceptor contribution to bradycardia will be made at even higher P_{a,O_2} during apnoeic asphyxia.

The present results suggest that in normal diving the cardiac response to submergence could be the expression of input from three groups of receptors. Muskrats actively close their external nares during unrestrained dives so nasal receptors cause both apnoea and pronounced bradycardia. Apnoea occurs in the expiratory phase of the respiratory cycle and, early in the dive, air is frequently released from the lungs. This lung deflation also provokes bradycardia but the degree of interaction between nasal receptor input and deflation in initiating bradycardia in normal diving is not clear. Under experimental conditions there is little doubt that withdrawal of lung receptor input enhances the cardiac response to nasal stimulation and the response is reduced by lung deafferentation. On the other hand, bradycardia provoked by nasal water flow is significantly greater than that occuring in unrestrained dives suggesting that interaction occuring in the laboratory is not mirrored in the field. After some 20 sec under water P_{a,O_2} will reach 8 kPa and this level of carotid body chemoreceptor input is known to cause bradycardia. We have not shown whether this input intensifies the existing diving bradycardia although, in voluntary dives of more than 5 sec, heart rate declines throughout the underwater period. Baroreceptors in muskrats also cause bradycardia in response to adrenaline-induced increases in blood pressure but we have been unable to show a role for them in either the cardiac or even blood pressure responses to diving.

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