Pflügers Arch. 366, 95–99 (1976)

Pflügers Archiv
European Journal of Physiology

The Effects of Asphyxia on Afferent Activity Recorded from the Cervical Vagus in the Duck

OWEN S. BAMFORD and DAVID R. JONES

Department of Zoology, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

Summary. Recordings were made of nervous activity from duck arterial chemoreceptors, arterial baroreceptors and pulmonary receptors during steady-state conditions (normoxic normocapnia, hypoxia, and hypercapnia) and apnoeic asphyxia. Arterial chemoreceptors were stimulated by hypoxia and intra-arterial KCN injection and showed an increasing discharge throughout asphyxia. During the first 2 min of asphyxia the time course of the development of asphyxic bradycardia paralleled that of the increase in arterial chemoreceptor discharge. Arterial baroreceptors discharged at a constant latency from the heart beat when mean arterial pressure was constant, while a drug-induced increase in mean arterial pressure was associated with a reduced latency and increased baroreceptor activity per heart-beat. During asphyxia mean arterial pressure often rose so that, despite the effect of bradycardia, baroreceptor activity per heart-beat and activity per unit time increased. Pulmonary receptors showed a linear relationship (negative slope) between discharge rate and % CO_2 in inspired air and usually stopped firing in apnoeic asphyxia. The initiation and maintenance of diving bradycardia are discussed in terms of these results.

Key words: Vagal activity – Chemoreceptor – Baroreceptor – Pulmonary receptor – Apnoea – Bradycardia – Ducks.

INTRODUCTION

When submerged, diving birds and mammals exhibit apnoea, bradycardia and increased peripheral vascular resistance. These reflexes are known collectively as the diving response and effectively reduce oxygen consumption of those tissues not susceptible to damage by oxygen deprivation, diverting the blood oxygen to the heart and central nervous system. Although the initiation of the response pattern has been the subject of much research and controversy, the maintenance of the diving responses has received less attention.

In ducks, the cardiovascular responses seen in laboratory diving experiments are responses to apnoea, and it is therefore the maintenance of apnoea that is crucial to the expression of the diving response (Butler and Jones, 1968; Bamford and Jones, 1974). However, during apnoea there is a progressive hypoxic hypercapnia, so chemoreceptor reflexes should provide a steadily increasing respiratory drive. Moreover, the trigeminal and glossopharyngeal inputs that normally inhibit breathing at the initiation of a dive are known to be ineffective after 100 s of apnoea (Bamford and Jones, 1974). After this time the duck apparently has a strong drive to breathe from chemoreceptor reflexes, and no remaining reflex inhibition from glottal or nasal receptors. Since it has been shown that ducks can endure submersion for at least 13 min (Pickwell, 1968), the possibility exists that chemoreceptor stimulation of breathing is somehow suppressed during a dive, either by decreasing receptor sensitivity or by reducing the gain of the reflex. It has also been suggested in mammals that pulmonary receptors may be important in maintaining apnoea through the Hering-Breuer reflex (Irving, 1937). While this suggestion has not been made for birds, a Hering-Breuer reflex does occur in ducks and could make some contribution to maintaining apnoea if the pulmonary receptors continued to discharge.

Recordings have been made of activity from avian arterial chemoreceptors (Bouverot and Leitner, 1972) and arterial baroreceptors (Jones, 1973), showing these receptors to have much the same properties as their mammalian counterparts. Avian pulmonary afferents have been studied extensively and the majority have been shown to be sensitive to CO_2 and not mechanical deformation (Fedde et al., 1974) producing a burst of activity on inspiration as air low in CO_2

flows through the lung. To date no recordings have been made of activity from any of these receptors during asphyxia. To investigate any change in receptor characteristics, recordings were made from these three classes of receptor under conditions of normoxic normocapnia, hypoxia, hypercapnia, hypertension, and during apnoeic asphyxia.

METHODS

Experiments were performed on 33 ducks, ranging in weight from 0.9–2.5 kg. Ducks were sedated by injection of urethane (100 mg/kg, i.m.) and all preparative operations, which were of a superficial nature, were done after first infiltrating the area of incision with local anaesthetic (Xylocaine" 2%, Astra-Hewlett Ltd.). The trachea was exposed by a midcervical skin incision and cannulated with a soft PVC tube for artificial ventilation. In ducks where unidirectional artificial ventilation was to be used, the interclavicular air sac was opened and a soft rubber tube sutured into the air sac. An ulnar vein and sciatic artery were cannulated with PE 90 and PE 160 tubing respectively, the cannulae being filled with heparinised saline (40 i.u./ml). The arterial cannula was used for measurement of arterial blood pressure using a Hewlett-Packard 267BC or Statham P23Gb transducer, blood samples being taken through a side-arm, while the venous cannula was used for administration of drugs. Tracheal air flow was measured with a pneumotachograph connected to the tracheal cannula. EKG was monitored via two copper wires, one inserted into a leg and the other into the opposite side of the neck.

After implantation of all cannulae, the duck was paralysed by injection of 1 mg/kg curare ('Tubarine', Burroughs Wellcome Ltd.) and ventilation taken over by either a Harvard small-animal respirator or by constant flow of humidified air + 4% CO_2 which entered by the tracheal cannula and left through the tube in the interclavicular air sac. In both cases body temperature was monitored by a rectal thermometer and maintained at 41±1° C with an infra-red lamp. With the duck in the prone position, an incision was made in the dorsal skin of the neck, under local anaesthetic, and a skin flap pulled out to expose the vagus nerve and jugular vein, usually on the left side. Under a binocular microscope the nerve was freed from its attachment for a length of 1–2 cm and a nickel base plate inserted under the nerve and fixed in position. The skin flap was filled with a pool of warm mineral oil. The initial level of sedation of the bird was maintained by further injections of urethane (i.v.).

Using fine forceps the nerve was de-sheathed over a short length. A small cut was made into the nerve at the cranial end of the de-sheathed portion and a slip of nerve was peeled back distally and placed on a pair of platinum hook electrodes. Recording and amplification was conventional, a rate-meter being used for display. The nerve slip was further dissected to obtain strands containing activity from sufficiently few fibres so that action potentials from the different fibres could be reliably separated on the basis of spike height, using a window discriminator (Frederic Haer & Co. Ann Arbor, Mich., U.S.A.). The output pulses from the discriminator were counted, using standard software, on a Digital Lab-8e computer. Nerve activity in the slips was examined for arterial chemoreceptor, arterial baroreceptor and pulmonary afferent activity, using the following criteria:

Arterial Chemoreceptors. Slow random discharge, greatly increased following intravenous injection of 100 µg KCN with a short latency (under 5 s), and increasing discharge rate in hypoxia. Unaffected by changes in arterial pressure.

Arterial Baroreceptors. Firing in short bursts, with constant latency from EKG. Discharge increased by an elevation in arterial blood pressure, whether induced by intravenous adrenalin (5–8 µg/kg) or occurring spontaneously during asphyxia.

Pulmonary Afferents. Discharging in regular bursts with lung inflation, with the discharge reduced or abolished by an increase in CO_2 in the ventilating mixture.

Discharges identified as belonging to one of these three classes of receptor were recorded while the animal was subjected to different levels of hypoxia or hypercapnia, asphyxia, and hypertension. Hypoxia and hypercapnia were induced by appropriate gas mixtures, asphyxia by stopping ventilation in expiration, and hypertension by intravenous injections of adrenaline. Arterial pressure, EKG, tracheal air flow, and nerve discharge were recorded on a seven-channel FM tape recorder (Hewlett-Packard 3907C) for later analysis. A spare channel on the tape carried an event marker.

In a few experiments, an arterio-venous loop was established from the arterial to the venous cannula. This loop contained a Beckman oxygen macroelectrode in a curvette maintained at body temperature and was used to monitor Pa_{O_2} during apnoea. The blood and electrode were maintained at 41±0.2° C and the electrode was calibrated at this temperature with air-equilibrated duck blood and air-equilibrated saline and nitrogen gas. The same reading was obtained for air-equilibrated duck blood and air-equilibrated saline at body temperature. The 90% response time was 3–5 s for the oxygen electrode and circulation time through the cannula-cuvette-cannula system was of the order of 1–2 s in the resting animal. Output of the oxygen electrode was unaffected by positive pressure and was independent of flows from 9–50 ml/min. In most cases, however, blood gases and pH were measured conventionally from samples taken from the arterial cannula, using a Radiometer PHM 71 meter and BMS 3 system at 41° C. Blood gas tensions were measured periodically under control conditions (normoxic normocapnia), during hypoxia, hypercapnia, and after 150 s of asphyxia.

In experiments in which unidirectional ventilation was used, the percentage of O_2 and CO_2 in the outflowing gas were monitored using Beckman F3 and LB 15 A analysers. Arterial blood gas tensions were controlled by adjustment of the gas flow rate and CO_2 content. When studying pulmonary receptors measurements of CO_2 content in the inhaled gas were also made. The gas analysis equipment was calibrated using precision gas mixtures.

RESULTS

Chemoreceptors. Eleven chemoreceptor fibres were identified by the established criteria. Generally, the activity was of low amplitude and single-fibre recordings were difficult to obtain, though in most cases the number of active fibres present in a nerve slip was small enough to allow discrimination between units on the basis of spike height. Activity from chemoreceptor fibres was found, as in mammalian chemoreceptors, to be random in that interval histograms of the discharges showed a Poisson distribution which was maintained over a wide range of mean rates of discharge provoked by changes in Pa_{O_2}. The relationship between Pa_{O_2} and discharge rate varied somewhat from one fibre to another but all fibres showed a marked increase in discharge rate when Pa_{O_2} fell below 50 torr. CO_2 stimulated the receptors,

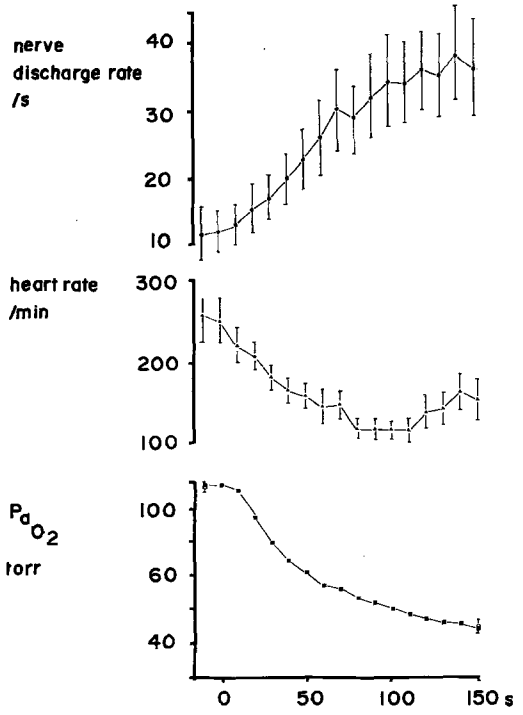

Fig. 1. Changes in arterial chemoreceptor activity, heart rate and Pa_{O_2} before and during asphyxia. The figure represents data from 11 ducks. The graphs show, from above down: mean nerve discharge rate/s; mean heart rate/min; and Pa_{O_2} (torr). In the bottom graph, the solid squares represent the mean of data from 2 experiments in which Pa_{O_2} was monitored continuously and the open squares represent mean values of Pa_{O_2} from blood samples taken, at the time indicated, from 11 animals. The period of asphyxia started at time 0 s

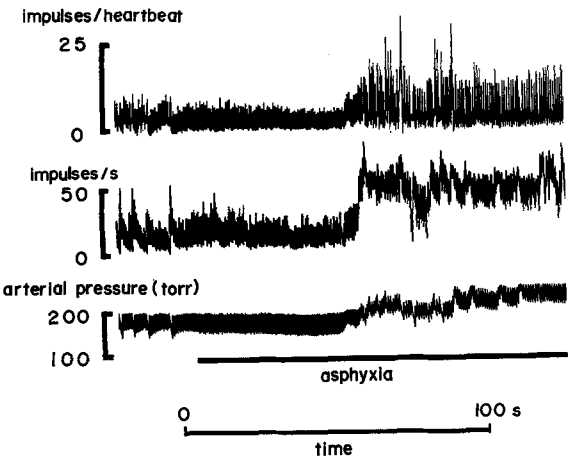

Fig. 2. Changes in activity in a single baroreceptor fibre before and during a period of asphyxia. Traces show, from above down; number of impulses per heartbeat; rate-meter output showing number of impulses/s; arterial pressure (torr); time (s). The solid horizontal bar indicates the onset of the asphyxic period

though too few data points were obtained for a quantitative assessment of the relationship between Pa_{CO_2} and discharge rate.

During asphyxia chemoreceptor fibres increased their activity. Generally there was a lag of some 10–20 s between the onset of asphyxia and the first detectable increase in discharge rate, after which the rate increased from 10 to 30 ± 4 impulses/s by the end of the first minute (Fig. 1). Simultaneous recording of the Pa_{O_2} showed that for the first 2 min of asphyxia, the curve of chemoreceptor discharge rate was the inverse of the curve of Pa_{O_2}. Thus the relationship between Pa_{O_2} and discharge rate was approximately linear over this range. Furthermore, the heart rate depression followed the same time course, so there was a linear relationship (negative slope) between heart rate and chemoreceptor discharge rate. Under the conditions of these experiments bradycardia tended to break after the asphyxic blood sample was taken and the heart rate then became very unstable, such that at the end of the asphyxic period the heart rate was not significantly different from control values and the relationship between Pa_{O_2}, chemoreceptor discharge, and heart rate was lost.

Baroreceptors. Recordings were made from 12 baroreceptor fibres under normal conditions, during adrenalin-induced elevation of arterial blood pressure, and during asphyxia. At normal arterial pressures the time between the QRS complex and individual spikes of the baroreceptor discharge was constant to within less than 5 ms, but during adrenalin-induced elevation of arterial pressure the latency became shorter and more variable while the discharge was less precisely repeated one beat to the next and contained many more action potentials. At very high arterial pressures the discharge became continuous, though the frequency was still modulated by the pressure pulse.

Under our experimental conditions arterial pressure usually increased during asphyxia and there was a corresponding increase in the number of impulses per heart beat (Fig. 2). Asphyxic bradycardia was often insufficient to counteract the increase in number of spikes per heart beat, and, consequently, the total baroreceptor activity per unit time also increased during the asphyxic period.

Pulmonary Receptors. Discharges were recorded from 17 pulmonary receptor fibres. In a breathing duck there was little activity during the greater part of the respiratory cycle. Nerve discharge started just after the onset of inspiration and increased steadily to terminate abruptly at peak inspiration. During artificial tidal ventilation, the phase relation between the ventilation cycle and discharge altered, probably because of the altered pattern of air flow through the lungs

in positive-pressure artificial ventilation compared with spontaneous breathing. The number of spikes recorded at each inflation was inversely proportional to the amount of CO_2 in the inspired gas mixture. The activity of most pulmonary receptors ceased during asphyxia, although a few receptors maintained a low level of activity during some or all of the asphyxic period. The first inflation, or the onset of unidirectional ventilation, terminating asphyxia was always associated with a short-lived increase in discharge to above normal levels, even though the ventilation rate and volume were unchanged from before asphyxia. During unidirectional ventilation pulmonary receptors gave a steady regular discharge at a rate inversely proportional to the level of CO_2 in the ventilating gas stream.

DISCUSSION

The cardiovascular responses to apnoeic asphyxia are very different from those provoked by breathing gas mixtures yielding Pa_{O_2} and Pa_{CO_2} similar to that found at the end of a period of asphyxia (Butler and Taylor, 1973). However, the present results show that avian cardiovascular receptors behave during apnoeic asphyxia in a manner consistent with their responses to various levels of Pa_{O_2} or blood pressure during eupnoea or hyperpnoea. Therefore, the special cardiovascular adjustments to apnoeic asphyxia cannot be explained in terms of modified receptor responses since there appeared to be little or no change in threshold or sensitivity of these receptors.

The arterial chemoreceptors studied behaved with a high degree of consistency, and the increase in discharge frequency was similar during normocapnic hypoxaemia and asphyxia at the same Pa_{O_2} indicating a low sensitivity to CO_2. Unfortunately, a quantitative assessment of the effect of hypercapnaemia on receptor discharge was not made, although it appeared that CO_2 sensitivity over the range of 40–60 mm Hg Pa_{CO_2} at normal Pa_{O_2} was not high. In this respect avian carotid body chemoreceptors are very similar to those of the mammal (Biscoe et al., 1967). It was demonstrated by Jones and Purves (1970) that chemodenervation abolished diving bradycardia suggesting that bradycardia was largely a chemoreceptor driven response to progressive hypoxaemia. Certainly the correlation shown in Figure 1 between the extent of asphyxic bradycardia and chemoreceptor discharge rate would appear to support this conclusion. The instability of heart rate shown late in asphyxia was probably an artefact and is unusual in our experience. We do not consider that this result argues against the role of the chemoreceptor discharge in causing asphyxic bradycardia.

On the other hand there is a possibility that diving bradycardia is a secondary response to a primary chemoreflex vasoconstriction, as suggested by Blix et al. (1974). Angell James and Daly (1969) showed such a vasoconstriction in dogs, in the absence of lung movements. Where the arterial pressure rises during asphyxia, as during the present work, it is feasible that baroreceptor reflexes may help to maintain bradycardia. However, during a dive arterial pressure in an intact duck tends to fall slightly. It has also been shown that after denervation of the baroreceptors the increase in peripheral resistance is smaller, but diving bradycardia in unaffected (Jones, 1973). Thus it is likely that in a dive the heart rate is largely under the control of the chemoreceptors, but a secondary reflex via the baroreceptors cannot at present be ruled out.

The pulmonary afferents examined all showed a more or less complete inhibition of activity during apnoea and hence cannot be involved in a Hering-Breuer reflex. While it could be argued that they have no function during apnoea, this may be a simplistic view of their normal function. Cohen (1964) showed that pulmonary feedback was necessary for the normal pattern of respiratory chemosensitivity to be shown in the cat, and that when pulmonary afferent traffic was abolished by bilateral vagotomy the normal stimulatory effect of CO_2 on respiratory rate became an inhibitory effect. Evidence exists for a considerably more dramatic reversal of respiratory chemosensitivity in ducks during apnoea (Jones and Bamford, 1976). Thus the lack of respiration-related afferent traffic may be important in changing the responses of the animal to variations in blood gases, though the mechanism by which such a change could occur is not understood at present.

From the present study it is clear that there is little or no reduction in sensitivity of the arterial chemoreceptors during asphyxia. It might therefore be expected that an intense respiratory drive would exist during asphyxia, but it has been shown repeatedly that submerged ducks can remain apnoeic for extended periods without any apparent respiratory efforts. Under these conditions there must therefore be a reduction in the sensitivity of the respiratory chemoreflexes, and although the mechanism of this alteration in sensitivity is not known, it is likely that the withdrawal of rhythmic pulmonary afferent information is involved.

Acknowledgements. Both authors held fellowships from the Killam Foundation during the period in which this work was carried out. The work was supported by a grant-in-aid from the B.C. Heart Fund.

REFERENCES

Angell James, J. E., Daly, M. de B.: Cardiovascular responses in apnoeic asphyxia; role of arterial chemoreceptors and the modification of their effects by a pulmonary vagal inflation reflex. J. Physiol. (Lond.) **201**, 87–104 (1969)

Anrep, G. V., Pascual, W., Rössler, R.: Respiratory variation of the heart rate: I-The reflex mechanism of the respiratory arrhythmia. Proc. roy. Soc. B **119**, 191–207 (1936)

Bamford, O. S., Jones, D. R.: On the initiation of apnoea and some cardiovascular responses to submergence in ducks. Respir. Physiol. **22**, 199–216 (1974)

Bamford, O. S., Jones, D. R.: Respiratory and cardiovascular interactions in ducks: the effect of lung denervation on the initiation of and recovery from some cardiovascular response submergence. J. Physiol. (Lond.). In press

Biscoe, T. J., Sampson, S. R., Purves, M. J.: Stimulus response curves of single carotid body chemoreceptor afferent fibres. Nature (Lond.) **215**, 654–655 (1967)

Blix, A. S., Gautvik, E. L., Refsum, H.: Aspects of the relative roles of peripheral vasoconstriction and vagal bradycardia in the establishment of the "diving reflex" in ducks. Acta physiol. scand. **90**, 289–296 (1974)

Bouverot, P., Leitner, L.-M.: Arterial chemoreceptors in the domestic fowl. Respir. Physiol. **15**, 310–320 (1972)

Butler, P. J., Jones, D. R.: Onset of and recovery from diving bradycardia in ducks. J. Physiol. (Lond.) **196**, 255–272 (1968)

Butler, P. J., Taylor, E. W.: The effect of hypercapnic hypoxia, accompanied by different levels of lung ventilation, on heart rate in the duck. Respir. Physiol. **19**, 176–187 (1973)

Cohen, M. I.: Respiratory periodicity in the paralyzed, vagotomised cat: hypocapnic polypnea. Amer. J. Physiol. **206**, 845–854 (1964)

Fedde, M. E., Gatz, R. N., Slama, H., Scheid, P.: Intrapulmonary CO_2 receptors in the duck: I. Stimulus specificity. Respir. Physiol. **22**, 99–114 (1974)

Irving, L.: The respiration of beaver. J. cell. comp. Physiol. **9**, 437–451 (1937)

Jones, D. R.: Systemic arterial baroreceptors in ducks and the consequences of their denervation on some cardiovascular responses to diving. J. Physiol. (Lond.) **234**, 499–518 (1973)

Jones, D. R., Bamford, O. S.: Open-loop respiratory chemosensitivity in chickens and ducks. Amer. J. Physiol. **230**, 861–867 (1976)

Jones, D. R., Purves, M. J.: The carotid body in the duck and the consequences of its denervation upon the cardiac responses to immersion. J. Physiol. (Lond.) **211**, 279–294 (1970)

Pickwell, G. V.: Energy metabolism in ducks during submergence asphyxia; assessment by a direct method. Comp. Biochem. Physiol. **27**, 455–485 (1968)

Received May 26, 1976