On the cardioinhibitory role of water immersion *per se* in ducklings (*Anas platyrhynchos*) during enforced submersion

DAVID R. JONES AND PATRICK J. BUTLER

The University of British Columbia, Department of Zoology, 6270 University Boulevard, Vancouver, B.C., Canada V6T 2A9

and

Department of Zoology and Comparative Physiology, The University of Birmingham, P.O. Box 363, Birmingham, United Kingdom B15 2TT

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Experiments were done to clarify the contribution of water immersion *per se* to the cardiac chronotropic response to enforced submergence in ducklings. Four protocols were used in which one or more of the factors claimed to affect diving heart rate were controlled. Heart rate fell rapidly in the first few seconds of submergence in all protocols. This initial fall was unaffected by changes in head position or by breathing 100% oxygen for 5 min before diving. Also, sudden withdrawal of input from lung receptors or cessation of activity in central respiratory neurones did not affect onset of diving bradycardia. We conclude that about one-third of diving bradycardia in ducklings is caused by water immersion *per se*.

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Des expériences ont permis de faire la lumière sur le rôle de l'immersion dans l'eau *per se* sur la réaction cardiaque chronotrope chez des canetons en immersion forcée. Quatre protocoles expérimentaux permettaient de contrôler l'un ou plusieurs des facteurs soupçonnés d'affecter le rythme cardiaque pendant la plongée. Le rythme cardiaque a ralenti rapidement durant les premières secondes d'immersion dans toutes les expériences. Cette chute initiale n'est pas affectée par la position de la tête ni par la respiration d'oxygène à 100% durant les 5 premières minutes précédant la plongée. De plus, un retrait subit de l'action des récepteurs pulmonaires ou la cessation de l'activité des neurones respiratoires centraux n'ont pas d'effet sur l'initiation de la bradycardie de plongée. En conclusion, environ un tiers de la bradycardie de plongée est causé par l'immersion *per se*.

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Introduction

A number of investigators have claimed that part of the bradycardia in response to forcible submersion in ducks is due to a specific immersion reflex, triggered by water in contact with the head or internal respiratory passages (Andersen 1963*a*, 1963*b*, 1963*c*; Feigl and Folkow 1963; Folkow *et al.* 1967; Rey 1971; Blix *et al.* 1975). This claim is supported by observations that an initial rapid decline in heart rate, accompanied by vasoconstriction, occurs on submergence even when ducks are artificially ventilated with air (Feigl and Folkow 1963; Blix *et al.* 1975). On the other hand, this has not been seen by all investigators (Huxley 1913; Lombroso 1913; Reite *et al.* 1963; Cohn *et al.* 1968; Bamford and Jones 1974) and, when it is, it is usually associated with high predive heart rates and could therefore be an artifact of the experimental regime (Jones, Milsom, and Gabbott, in preparation). However, ducklings (1–3 days old) have extremely high resting heart rates and recent work (West 1981) suggests that a specific immersion reflex, if it exists, is likely to be more obvious in ducklings than adults. Consequently, the present experiments were designed, using ducklings, to elucidate the role of immersion *per se* to the cardiac chronotropic response to submergence.

Methods

The experiments were done on eight ducklings varying in age from 1 to 3 days and in mass from 50 to 90 g. Heart rate was obtained from the electrocardiogram, recorded bipolarly, and breathing frequency from an impedance pneumogram recorded between electrodes on either side of the chest. After intubating the trachea, rhythmic artificial ventilation was performed using a positive pressure pump set to deliver a volume which gave the same deflection on the pneumogram as was obtained when the duck breathed spontaneously. The pump rate was adjusted to match the normal breathing frequency. Unidirectional ventilation was performed by passing air, at a known flow rate, down the tracheal tube to exit through a tube penetrating the wall of the interclavicular air sac. The tracheal tube was removed and the one in the interclavicular air sac clamped off when returning the duck to spontaneous breathing.

Ducks were restrained on an operating table, ventral side down, and dived into the mouth of a filter funnel. All dives (maximum duration of 30 s) involved forcible immersion of the head so we attempted to identify and control all other factors contributing to the cardiac chronotropic response to

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TABLE 1. The presumed effect of each protocol on a number of factors which may contribute to the initiation of the cardiac chronotropic response to submergence

Protocol	Position of the head	Water immersion	Activity in central respiratory neurones	Feedback from pulmonary afferents	Blood oxygen	Blood carbon dioxide
1	△	Yes	$\Delta^{\times}$	$\Delta^{\times}$	Δ^{-}	Δ^{+}
2	○	Yes	$\Delta^{\times}$	$\Delta^{\times}$	Δ^{-}	Δ^{+}
3	○	Yes	$\Delta^{\times}$	$\Delta^{\times}$	○	$\Delta^{+(?)}$
4						
Rhythmic artificial ventilation	○	Yes	$\Delta^{\times(?)}$	○	○	○
Unidirectional artificial ventilation	○	Yes	○	○	○	○

NOTE: △, change; ○, no change; +, rises; −, falls; ×, stops.

submergence using the following protocols, applied to all ducks in random order (Table 1).

Protocol 1

This protocol involved a dive with a change in head position; the head was gently lowered into the mouth of the funnel which was full of water. Releasing the head allowed the duckling to surface at the end of the dive.

Protocol 2

In this and all remaining protocols the head was held into the mouth of the empty funnel, by tape, for several minutes before a dive. Masking tape was used to occlude most of the mouth of the funnel and air was blown in through the spout. The dive was effected by turning off the air flow and substituting water flow through the spout until water completely covered the duckling's head. The funnel was drained through the spout for emergence.

Protocol 3

The animal was set up as in protocol 2 except that 100% O_2, instead of air, was flushed through the funnel and breathed by the duckling for 5 min predive.

Protocol 4

This protocol involved dives with artificial ventilation; the duckling was positioned as in protocol 2 but either rhythmic or unidirectional artificial ventilation, with air or 100% oxygen, was started some 1–2 min predive and continued throughout the dive. The unidirectional air flow rate was high enough to prevent resumption of spontaneous breathing by the animal for at least 10 s after it was suddenly stopped. Thus it is assumed that there was little or no rhythmic central respiratory activity during unidirectional air flow. Rectal temperature was unchanged by artificial ventilation. There was no significant difference between the heart rates during dives with rhythmic or unidirectional ventilation, either with air or 100% oxygen, so all these results were combined (Table 1).

Statistical analysis of data

In the text and figures numerical values, when referring to determinations of variables in a group of animals, are given as means ± SE of *n* observations on eight animals. Data from the various groups, in each series of experiments, were compared at each sampling time using a one-way analysis of variance (ONEWAY, SPSS: Nie *et al.* 1975). Comparisons of data within a group were conducted using a two-factor analysis with repeated measures over time (ANOVAR, SPSS). In the case of significant F values, pair-wise comparisons of means were done with either Scheffe's method (Scheffe 1959) or the Neuman–Keuls test (Winer 1971). $P < 0.05$ was taken as the fiducial limit of significance for differences between means.

Results and discussion

Predive heart rates with all four protocols were similar. There were no significant differences between diving heart rates in protocols 1 and 2 at any time, indicating a lack of any effect on diving bradycardia of the positional change (and handling) involved in the dive in protocol 1 (Table 1). Heart rates in dives done under protocol 3 were significantly above those in protocol 2 after 15 s submergence (Fig. 1) although this difference disappeared after 30 s submersion. The difference must be due to the effect of chemoreceptor input on heart rate in protocol 2 which was reduced, by breathing 100% O_2 predive, in protocol 3. Heart rates in protocol 4 (artificial ventilation through the dive) were significantly above those in protocol 2 after 5 s submergence and at all later dive times in all protocols. The similarity between heart rates in protocols 3 and 4 after 5 s submergence (Fig. 1) indicates that the sudden withdrawal of input from pulmonary receptors or cessation of activity in central respiratory neurones does not influence the onset of diving bradycardia (Table 1). In the first 5 s of submergence, the fall in heart rate in protocol 3 (14 ± 3 beats min^{-1} s^{-1}, n = 16) was not significantly different from that in protocol 4 (11.5 ± 2.6, n = 20) and, since chemoreceptors could not have contributed to bradycardia at this stage, must have been caused by the action of water immersion *per se* (Table 1 and Fig. 1). In the first 5 s of the dive, heart rate fell about 15–20% of predive

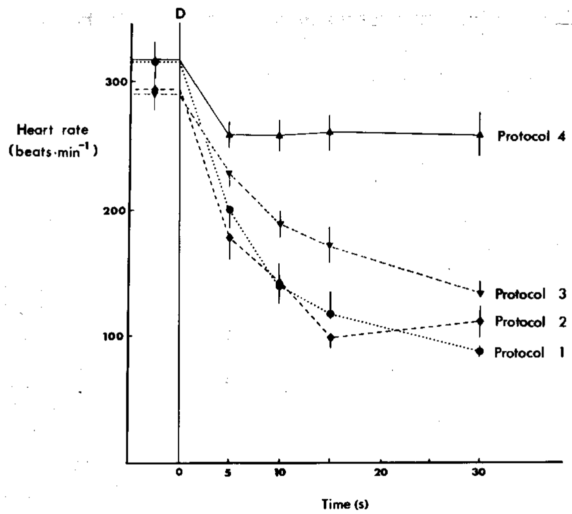

FIG. 1. Heart rates (beats per minute) ± SE of the mean during enforced submergence, using protocols 1 to 4 described in the text. The dives started at time 0, indicated by the vertical line marked D. Values to the left of this line are predive heart rates. n values for protocols 1 to 4 were 12, 16, 16, and 20 respectively.

rate which represents a contribution to bradycardia, developed after 30 s submergence following breathing air predive (protocol 2), of 20 to 30%. Since heart rates fell at a much faster rate in the first 5 s of dives done under protocols 1 and 2 (23 ± 4 beats min^{-1} s^{-1}, $n = 28$), then even in this short period, chemoreceptor input reinforces the effects of water immersion on heart rate (Fig. 1). That blood oxygen will fall rapidly in diving ducklings is suggested by the short underwater endurance of ducklings compared with adults (West 1981).

Hence, in ducklings, about one-third of the bradycardia during enforced submersion is due to an immersion reflex, the remainder being caused by stimulation of chemoreceptors with hypoxic and hypercapnic blood as in adults (Jones and Purves 1970; Lillo and Jones 1982). However, in adults the cardioinhibitory effects of immersion only seem to be expressed at high heart rates. In a recent study, Jones *et al.* (in preparation) obtained a strong correlation between preimmersion heart rate and the amount by which heart rate changed in the first 12 s of a dive; only if preimmersion heart rates were above 188 beats min^{-1} was there any fall in heart rate early in the dive. Consequently, the evanescence of cardioinhibitory immersion reflexes is probably due to better experimental approach by some investigators than others. In adults with normal resting heart rates, no effects of immersion on bradycardia are apparent, whereas in adults with high predive heart rates and in ducklings, an immersion reflex can make an important contribution to the bradycardia seen during forced submersion of the head (Feigl and Folkow 1963; Blix *et al.* 1975; West 1981).

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