Effects of changes in peripheral and central P_{CO_2} on ventilation during recovery from submergence in ducks

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The effects of increases in arterial CO_2 tension at peripheral and central chemoreceptors on ventilation during recovery from submergence were studied using cross-perfusion techniques on unanaesthetized, White Pekin ducks. Immediately upon surfacing, under normal conditions, minute ventilation ($\dot{V}_E$) was elevated four to five times due to roughly equal increases in tidal volume (V_T) and breathing frequency (f). Tidal volume returned to resting levels far more rapidly than breathing frequency. If only a peripheral hypercapnia was allowed to develop during diving, it produced the same maximum ventilatory reponse upon surfacing but recovery was much quicker. Central hypercapnia interacted with the peripheral hypercapnia in an additive fashion. There is evidence to suggest that hypercapnia has a greater effect in increasing V_T, and hypoxia in increasing f, during the postdive recovery period. The prolonged tachypnea which normally persists after blood gas levels have returned to normal only occurs when hypoxia is allowed to develop during the dive.

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Les effets d'une augmentation de la pression artérielle du CO_2 dans les chémorécepteurs périphériques et centraux sur la ventilation après la plongée ont pu être étudiés chez des canards de Pékin non anesthésiés, grâce à des techniques de perfusion croisée. En conditions normales, dès le retour à la surface, la ventilation par minute ($\dot{V}_E$) augmente de quatre à cinq fois, ce qui correspond grosso modo à des augmentations équivalentes du volume courant (V_T) et de la fréquence respiratoire (f). Le volume d'air revient au niveau normal beaucoup plus rapidement que la fréquence respiratoire. S'il ne se produit qu'une hypercapnie périphérique au cours de la plongée, le retour en surface est caractérisé par la même réaction respiratoire maximale, mais la récupération est beaucoup plus rapide. Une hypercapnie centrale produit des effets qui s'ajoutent à ceux de l'hypercapnie périphérique. Il semble que l'hypercapnie soit surtout responsable de l'accroissement du volume V_T et que l'hypoxie soit surtout responsable de l'accroissement de la fréquence f durant la période de récupération qui suit la plongée. La tachypnée prolongée, qui persiste normalement après que les concentrations des gaz sanguins sont revenus à la normale, ne se produit que s'il y a hypoxie au cours de la plongée.

[Traduit par le journal]

Introduction

During apnoea which accompanies submergence in diving animals, blood becomes progressively hypoxic and hypercapnic. This condition is reversed rapidly by the profound hyperpnea which follows the termination of a dive. This ventilatory response is a well-known feature of the postdive recovery period and has been measured quantitatively in ducks by several investigators (Butler and Jones 1968; Bamford and Jones 1976; Lillo and Jones 1982). At present, however, little is known of the mechanisms involved in the initiation and control of this ventilatory response. In quiet, resting ducks, hypoxia produces a strong ventilatory drive acting almost exclusively via peripheral (carotid body) chemoreceptors (Jones and Purves 1970; Bouverot, Hill, and Jammes 1974). Hypercapnia, on the other hand, acts via both central chemoreceptors and carotid body chemoreceptors (Milsom *et al.* 1981; Tallman and Grodins 1982). In a recent study, Lillo and Jones (1982) demonstrated that although the magnitude of the postdive hyperpnea was related to the length of the dive and the magnitude of the blood gas changes which ensued, much (73%) of the postdive ventilatory response remained following carotid body denervation and yet did not seem to be very dependent on hypercapnia. Thus the primary stimulus which drives the postdive hyperpnea remains unclear. As a consequence, the present studies were undertaken using a very different technique (cross perfusion between pairs of animals) from that used by Lillo and Jones (1982) to reassess this situation with particular emphasis on the role peripheral and central CO_2 receptors may play in this process.

Materials and methods

Experiments were performed on White Pekin ducks weighing between 2.5 and 4.5 kg. All surgery was performed under general anaesthesia (pentobarbital sodium, 30 mg·kg^{-1}) 1–2 days before experiments were run. The procedure used for preparing pairs of birds for cross perfusion has been described previously (Milsom *et al.* 1981). In the experiments described here, cannulae were inserted and connected as shown in Fig. 1. The cannula labelled as arising from the donor was connected to an upstream ischiatic cannula (flow from ischiatic artery of

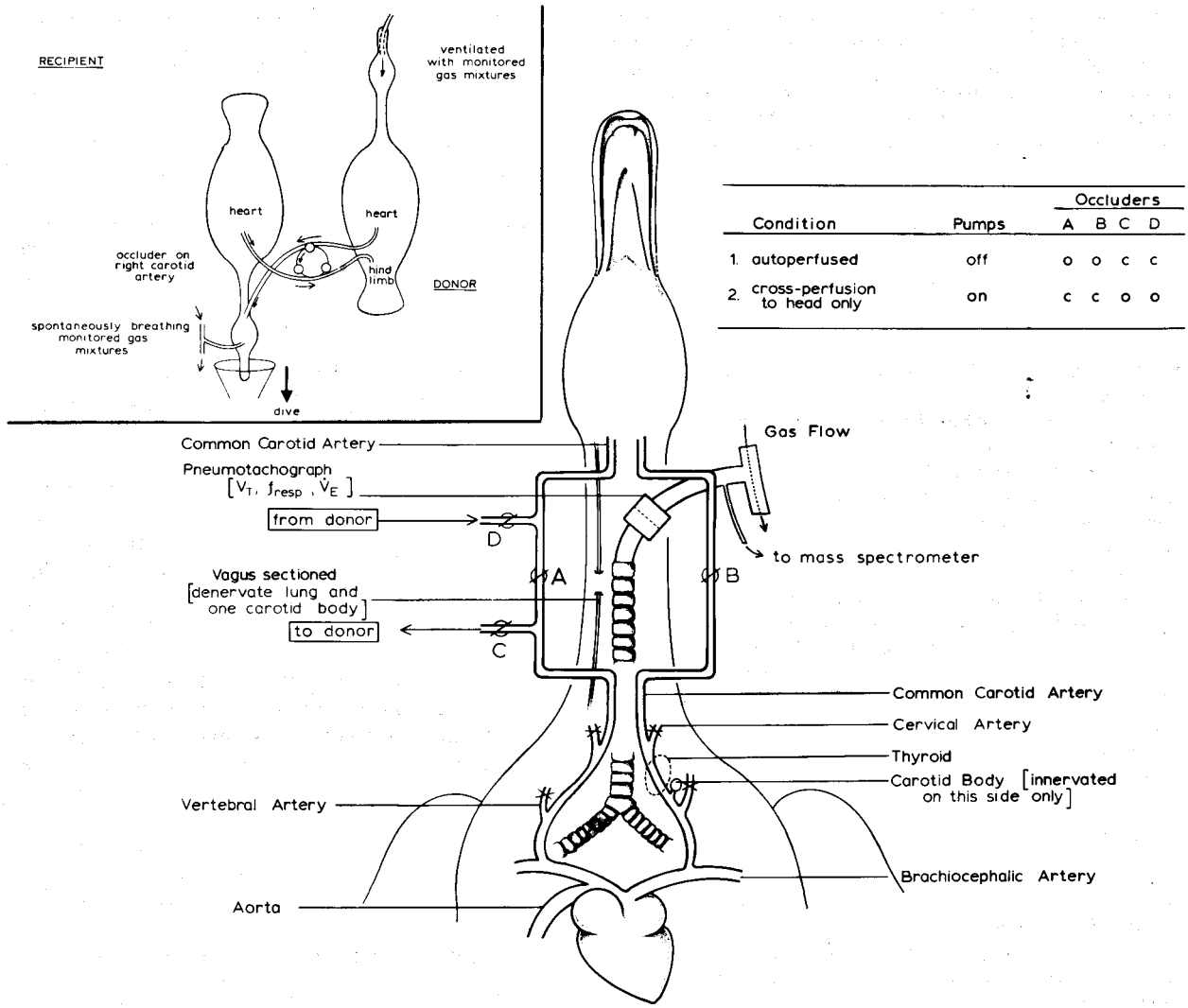

FIG. 1. Schematic diagram of preparation of recipient animal for cross perfusion. For further explanation, see text (in table: o, open; c, closed). Inset at upper left shows setup for cross perfusion.

donor to carotid artery of recipient); that labelled as going to the donor was connected to the downstream ischiatic cannula (flow from carotid artery of recipient to hind limb of donor) (Fig. 1). A pneumatic occluder was also placed on the intact (left) carotid artery (occluder B, Fig. 1). The flow through the two cannulae marked C and D was driven by a Harvard model 1210 peristaltic pump. With this preparation, it was possible to occlude blood flow at any of sites A, B, C, or D in Fig. 1.

The trachea was cannulated high in the neck and the distal end of the cannula was attached to a T connection. One arm of the T was open to atmosphere and the remaining arm was attached to a gas supply. Using a system of gas-flow meters, the composition of the gas flowing past the end of the cannula could be altered, controlling the composition of the inspired gas breathed by the recipient. Bipolar copper wire electrodes were attached to the recipient for monitoring the electrocardiogram. The vagus nerve was sectioned on the right-hand side, denervating one lung and one carotid body, as part of a separate set of experiments. All evidence indicates, however, that unilateral vagotomy does not alter normal ventilatory responses (Milsom *et al.* 1981).

Protocol

In all experiments the animals were unanaesthetized but lightly restrained, ventral side down, on operating tables. The donor duck was placed on unidirectional ventilation (gas administered via the trachea and vented via the interclavicular air sac) to allow rapid conditioning of arterial blood gases for cross perfusion to the recipient duck that breathed spontaneously.

Autoperfusion (perfusion pump off, occluders A and B open) (Fig. 1) represents the normal condition where the recipient animal is in total control of its own blood flow. Cross perfusion was established by occluding sites A and B and

switching on the perfusion pump (occluders C and D open) (Fig. 1). It has been shown previously (Milsom *et al.* 1981) that cross perfusion *per se* has no effect on the ventilatory responses of the recipient to changes in blood gas tensions. Once cross perfusion was established, 1000 IU heparin was administered every 2 h throughout the remainder of the experiments. Rectal temperatures of all birds were monitored constantly and maintained at 41 ± 1.0°C with heating lamps mounted above the birds.

The head of the recipient was fastened pointing downwards into a large funnel. Forced submergence was effected by filling the funnel with water and clamping the tracheal catheter after a short delay to allow the bird to exhale during submergence. Following 2 min of submergence, the tracheal cannula was reopened and the funnel was drained by removing a clamp from its spout.

The gas tensions of blood bathing the peripheral and central chemoreceptor groups at the start of the postdive recovery period were then varied. During normal, autoperfused dives, progressive hypoxia and hypercapnia develop at all receptor sites. If these animals were given 50% O_2 in air to breathe before diving, arterial P_{O_2} remained elevated throughout the dive and only progressive hypercapnia ensued. Once cross perfusion was established, the blood gas compositions at the central chemosensitive sites could be maintained independent of the blood gas composition in the periphery. In this way, postdive ventilatory responses could be studied in animals subject to: peripheral hypercapnia only (group I), peripheral and central hypercapnia (group II), and peripheral and central hypoxia and hypercapnia (group III). It should be noted, however, that peripheral blood gas changes developed progressively throughout the dive and were reversed during the recovery period, whereas central blood gas changes during cross-perfusion experiments were established during the predive period and maintained throughout the postdive recovery period. The level of central hypercapnia used in these experiments was in the range of that seen in ducks during prolonged dives (Lillo and Jones 1982).

At the end of the day's experiments the recipient animal was anaesthetized, placed on cross perfusion, and then the perfusion pump was turned off. Only if the recipient animal died within 1 min (as judged by respiratory failure) was cross perfusion considered to be supplying all of the recipient's cerebral blood flow and the experimental results deemed acceptable.

Measurements

Arterial blood pressure was monitored in the upstream segments of the carotid and ischiatic cannulae in the recipient and donor ducks, respectively, and perfusion pressure was measured in the cross perfusion cannula supplying the recipient (using Biotec BT-70 pressure transducers). When cross perfusion was established, flow was adjusted so that the perfusion pressure to the head of the recipient was equal to the arterial blood pressure of the recipient duck. Breathing was monitored in the recipient duck with a pneumotach attached to the tracheal cannula (Hewlett-Packard pneumotachograph No. A547). The pressure drop across the pneumotachograph during tracheal air flow was recorded with a Hewlett-Packard model 270 differential pressure transducer. The gas composition of all respiratory gases was monitored with a Centronic 200 MGA clinical mass spectometer. All signals were amplified using conventional means and the blood pressure, tracheal airflow, electrocardiogram, and O_2 and CO_2 compositions were displayed on a Technirite eight-channel thermal pen recorder writing on rectilinear coordinates. All signals were stored on an eight-channel FM tape system for later analysis at which time the tracheal air flow signal was fed through a Gould integrating preamplifier to give tidal volume.

Arterial blood samples taken immediately before the measurement of all respiratory variables in each experimental run were analyzed using an Instrumentation Laboratories IL micro-13 blood gas analyzer maintained at 41 ± 1°C.

All measurements are reported as the means ± SE.

Results

Under normal conditions (autoperfusion; animals breathing room air prior to diving (group III)) minute ventilation ($\dot{V}_E$) was elevated to three to five times resting values immediately following 2 min of submergence. These values were still elevated to two times resting values 1 min later (Figs. 2A, 3A, 3B; Table 1). Although all dives were invariably followed by this period of intense hyperpnea, the associated breathing pattern was not always uniform. On occasion, a breathing pattern consisting of rapid, shallow breaths began on surfacing and gradually gave way to slower, deeper breathing (Fig. 2B). In rare instances, animals did not resume breathing immediately on surfacing but eventually took one or two "exploratory" breaths before breaking into a pattern of rapid breathing (Fig. 2C). As a rule, however, animals began breathing immediately on surfacing with rapid, deep breaths (Fig. 2A). This was considered the normal response, and only postdive recovery periods exhibiting this pattern were used in the data analysis. With this pattern, the volume of the first inspiration often greatly exceeded the following expira-

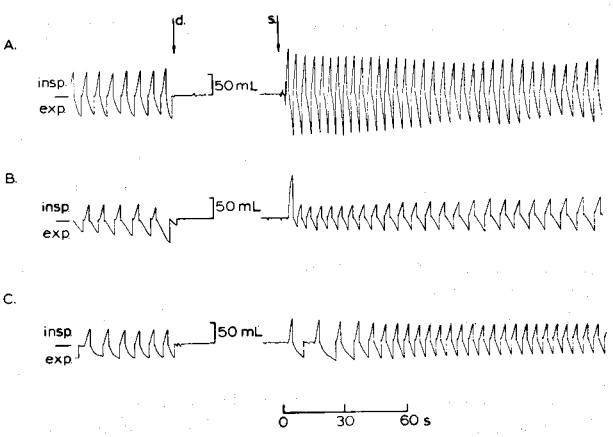

FIG. 2. Recordings of tidal volume during spontaneous breathing both before submergence (*d*) and following surfacing (*s*) to illustrate variations found in postdive breathing patterns (insp., inspiration; exp., expiration).

TABLE 1. Ventilatory values before submergence and during the 1st min following submergence

	Group I	Group II	Group III
V_T (mL)			
Predive	89±11	97±18	86±7
Recovery breath No. 1	194±19	214±23	168±20
2	126±15	138±14	131±13
3	119±16	139±15	128±13
4	104±12	133±15	122±12
5	106±16	131±17	120±11
30 s	103±14	117±13	111±9
60 s	95±14	114±14	108±10
f_{inst}(min^{-1})			
Predive	11±1.1	15±1.0	15±0.8
Recovery breath No. 1	16±2.0	22±2.7	29±1.8
2	15±1.4	20±1.5	26±1.6
3	16±1.5	21±1.2	25±1.1
4	16±1.1	21±1.2	25±1.1
5	14±1.2	21±1.3	26±1.1
30 s	13±0.9	19±1.7	24±1.0
60 s	12±0.7	16±1.4	21±0.6
$\dot{V}_{E_{inst}}$(mL·min^{-1})			
Predive	931±162	1410±160	1281±111
Recovery breath No. 1	3024±388	4191±250	4762±547
2	1814±171	2722±292	3317±344
3	1818±249	2859±319	3201±327
4	1554±115	2706±357	3021±288
5	1391±149	2765±399	3111±311
30 s	1225±135	2177±253	2725±277
60 s	1091±150	1735±227	2234±205
n	8	8	11

TABLE 2. Arterial blood gas values of blood perfusing the peripheral and central circulation before and immediately following sumergence

		Peripheral			Central		
Group	Condition	P_{O_2}	P_{CO_2}	pH	P_{O_2}	P_{CO_2}	pH
I	Predive	430.7±4.3	37.5±2.7	7.48±0.01	151.1±23.0	17.3±0.8	7.80±0.01
	Postdive	391.7±11.8	74.7±2.5	7.30±0.01	151.1±23.0	17.3±0.8	7.80±0.01
II	Predive	414.3±2.2	28.9±1.3	7.62±0.01	162.9±23.3	58.0±2.8	7.32±0.01
	Postdive	293.6±58.3	80.7±4.0	7.26±0.01	162.9±23.3	58.0±2.8	7.32±0.01
III	Predive	82.8±1.1	34.5±0.9	7.54±0.01	82.8±1.1	34.5±0.9	7.54±0.01
	Postdive	49.6±1.0	46.1±0.7	7.45±0.01	49.6±1.0	46.1±0.7	7.45±0.01

tion, restoring or elevating reserve volume. Both tidal volume (V_T) and breathing frequency (f) were elevated to roughly two times resting values with tidal volume returning to resting levels more rapidly than breathing frequency (Fig. 4, Table 1).

In group I animals, central blood gas levels were held constant at predive levels, via cross perfusion, throughout both the dive and postdive period (Table 2). Central P_{CO_2} was low to reduce any central stimulatory component to breathing. Furthermore, as the recipient animal was given 50% O_2 to breathe prior to diving, only a peripheral hypercapnia ensued during diving with no concomitant hypoxia. Because of the initial hyperoxia before the dive, $\dot{V}_E$ was reduced at this time (Table 1). Following the dive, however, $\dot{V}_E$ was still elevated to 3.5 times predive levels (Fig. 3A), although this

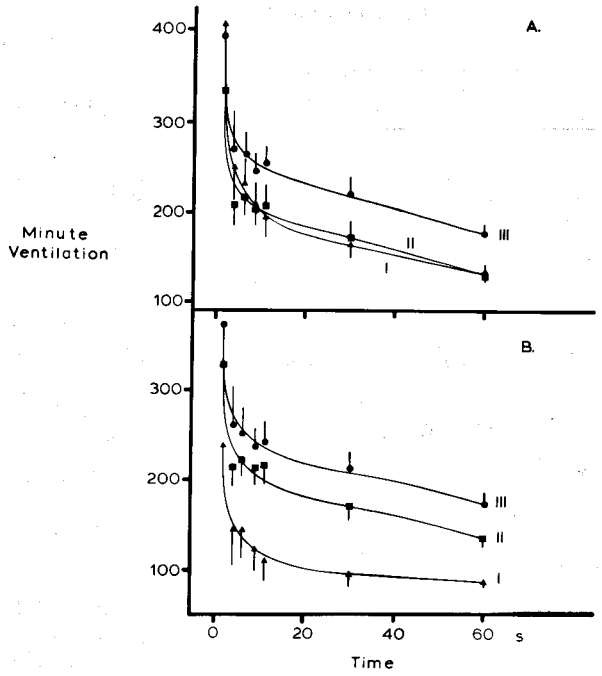

FIG. 3. Minute ventilation as a function of time following the end of 2 min of submergence in the various experimental groups. (A) The data are normalized to predive values for each group (predive values = 100%). (B) The data are all normalized to the group III (normal) predive values (for explanation of experimental groups, see text). Each value = (recorded value/Group III predive value) × 100.

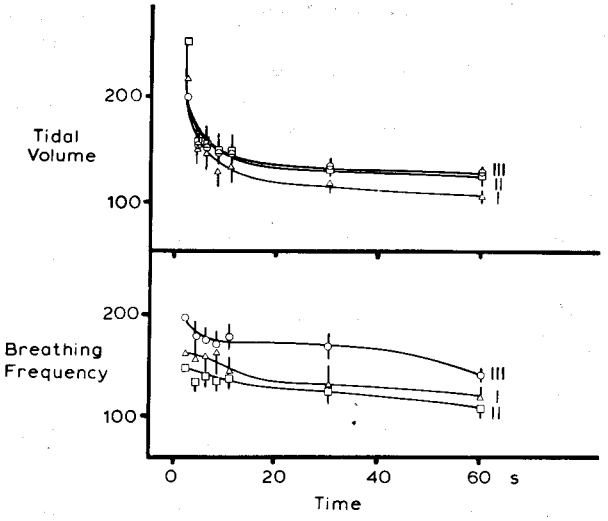

FIG. 4. Tidal volume (V_T) and breathing frequency (f) as functions of time following 2 min of submergence in the various experimental groups. All data is normalized to the predive values for each group.

represents only an increase of 2.5 times over the normal predive levels measured when the animals breathed air before the dive (Fig. 3B). This hyperpnea decreased rapidly and ventilation returned to predive levels in about 1 min (Fig. 3A, Table 1).

In group II animals, central P_{CO_2} was maintained at elevated levels throughout the experimental run (Table 2). The recipient animal was again given 50% O_2 to breathe prior to submergence so that only a peripheral hypercapnia developed during the dive. The initial central hypercapnia more than offset the peripheral hyperoxia and thus $\dot{V}_E$ was elevated slightly before diving (Table 1). Following 2 min of submergence, $\dot{V}_E$ was elevated to three times resting values with increases in V_T playing a proportionately greater role than increases in f (Figs. 3A, 4). This hyperpnea also decayed rapidly with $\dot{V}_E$ falling to near predive levels in approximately 1 min; that is, the peripheral hypercapnia was eliminated and breathing returned to near predive levels with the same time course as in the group I animals (Fig. 3A). This indicates that there was no interaction between the effects of CO_2 acting centrally and peripherally. Because of the maintained central hypercapnia, however, the level of minute ventilation recorded in the group II animals 1 min into the recovery period was still 1.5 times the level recorded in the normal animals before submergence (Fig. 3B). Even so, this level of $\dot{V}_E$ is still well below the level of hyperpnea present in the normal, group III animals, after 1 min of the recovery period (Figs. 3A, 3B).

Discussion

The ventilatory responses observed in the postdive recovery period in this investigation are in agreement with previous reports (Butler and Jones 1968; Bamford and Jones 1976; Lillo and Jones 1982). Predive ventilatory and blood gas values also compare favourably with resting values from other studies (Jones and Holeton 1972; Bouverot, Hildwein, and Le Goff 1974; Bouverot, Hill, and Jammes 1974; Bouverot *et al.* 1979; Kawashiro and Scheid 1975).

During the postdive hyperpnea seen under control conditions, the immediate increase in pulmonary ventilation was due to roughly equal changes in V_T and f. The increase in V_T returned to resting levels much more rapidly during the recovery phase than the increase in f, thus the relative role of frequency changes in the overall response increased with time. When the blood gas changes accompanying diving were confined to only a peripheral hypercapnia, the maximum ventilatory response on surfacing was unaltered. This confirms the work of Lillo and Jones (1982) suggesting that a $\dot{V}_E$ of approximately 4.5 $L \cdot min^{-1}$ may be the maximum possible in this species during recovery from submergence under the conditions of our experiments. The duration of the recovery phase, however, was now drastically reduced and the relative roles of changes in V_T and f in

producing the postdive hyperpnea were altered. Now increases in breathing frequency were reduced, and thus the increase in V_T played a much greater relative role in producing the postdive hyperpnea. This increase in the proportionate role of V_T in the postdive ventilatory response was further enhanced by central hypercapnia. This suggests that although hypercapnia and hypoxia both act to increase ventilation following diving, hypercapnia has a relatively stronger effect in increasing V_T while hypoxia acts primarily to increase breathing frequency as has been shown under predive, resting conditions (Bouverot, Hill, and Jammes 1974).

Following dives where the blood gas changes were confined to only a peripheral hypercapnia, ventilation did not return to predive levels for about 1 min suggesting that, for CO_2 at least, although the carotid bodies are not essential for the major portion of the postdive hyperpnea, they do make a significant contribution.

The response to peripheral hypercapnia is not altered by concomitant central hypercapnia. These two stimuli are simply additive. Because central hypercapnia was maintained throughout the postdive period in these experiments and could not be alleviated by the hyperpnea, it is not possible to quantify the role central hypercapnia plays in the overall response. The data do suggest, however, that it cannot be very large, for even with maintained central hypercapnia, minute ventilation 1 min into the recovery period was still well below the levels recorded at this time following a normal dive. These data suggest that hypoxia must be the major stimulus for the prolonged hyperpnea following normal diving. It must be remembered that following normal dives, blood gases are rapidly returned to normal (Butler and Jones 1971). Certainly, in this present study the peripheral CO_2 stimulus was alleviated in about 1 min. Thus the hyperpnea is prolonged long beyond the time when blood gases have returned to normal. In the work of Lillo and Jones (1982) denervation of the carotid bodies did not reduce the postdive ventilatory response greatly, leading to the conclusion that the major effects of hypoxia on this response must be mediated by some route other than the carotid body chemoreceptors. Their denervations did reduce the changes in tidal volume, however, but did not alter the frequency response. Thus it would appear that although any of hypoxia or hypercapnia acting via the carotid bodies, or hypercapnia acting via the central CO_2 chemoreceptors, will produce a maximum ventilatory response following diving, these stimuli and the responses they elicit are rapidly eliminated yet a prolonged hyperpnea remains. This prolonged hyperpnea is a hypoxic response due primarily, if not exclusively, to increases in breathing frequency and accounts for the continual increase throughout the recovery period in the relative role of changes in f over changes in V_T in the overall ventilatory response. The afferent limb of this response remains unknown. Thus, although the magnitude of the postdive hyperpnea is related to the length of the dive and the magnitude of the consequent blood gas changes, it cannot be completely accounted for by conventional chemoreceptor reflexes.

Acknowledgements

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BAMFORD, O. S., and D. R. JONES. 1976. Respiratory and cardiovascular interactions in ducks: the effect of lung denervation on the initiation of and recovery from some cardiovascular responses to submergence. J. Physiol. (London), **259**: 575–596.

BOUVEROT, P., D. DOUGUET, and P. SEBERT. 1979. Role of the arterial chemoreceptors in ventilatory and circulatory adjustments to hypoxia in awake Pekin ducks. J. Comp. Physiol. **133**: 177–186.

BOUVEROT, P., G. HILDWEIN, and D. LE GOFF. 1974. Evaporative water loss, respiratory pattern, gas exchange and acid base balance during thermal panting in Pekin ducks exposed to moderate heat. Respir. Physiol. **21**: 255–269.

BOUVEROT, P., N. HILL, and Y. JAMMES. 1974. Ventilatory responses to CO_2 in intact and chronically chemodenervated Pekin ducks. Respir. Physiol. **22**: 137–156.

BUTLER, P. J., and D. R. JONES. 1968. Onset and recovery from diving bradycardia in ducks. J. Physiol. (London), **196**: 255–272.

——— 1971. The effect of variations in heart rate and regional distribution of blood flow on the normal pressor response to diving in ducks. J. Physiol. (London), **214**: 457–479.

JONES, D. R., and G. F. HOLETON. 1972. Cardiac output of ducks during diving. Comp. Biochem. Physiol. A, **41**: 639–645.

JONES, D. R., and M. J. PURVES. 1970. The effect of carotid body denervation upon the respiratory response to hypoxia and hypercapnia in the duck. J. Physiol. (London), **211**: 295–309.

KAWASHIRO, T., and P. SCHEID. 1975. Arterial blood gases in undisturbed resting birds: measurements in chicken and duck. Respir. Physiol. **23**: 337–342.

LILLO, R. S., and D. R. JONES. 1982. Effect of cardiovascular variables on hyperpnea during recovery from diving in ducks. J. Appl. Physiol. **52**: 206–215.

MILSOM, W. K., D. R. JONES, and G. R. J. GABBOTT. 1981. On chemoreceptor control of ventilatory responses to CO_2 in unanaesthetized ducks. J. Appl. Physiol. **50**: 1121–1128.

TALLMAN, R. D., and F. S. GRODINS. 1982. Intrapulmonary CO_2 receptors and ventilatory response to lung CO_2 loading. J. Appl. Physiol. **52**: 1272–1277.