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The effect of brain transection on the response to forced submergence in ducks

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Abstract

The effect of brain transection at two levels on cardiovascular responses to forced submergence has been investigated in ducks. Compared with intact ducks, neither decerebration nor brain stem transection at the rostral mesencephalic (RM) level had any effect on development of diving bradycardia, or heart rate at the end of two-min dives. Arterial blood pressure was maintained in brain transected ducks as well as in intact ducks. Furthermore, end-dive arterial blood gases and pH were also similar in intact and brain transected ducks confirming that the oxygen sparing cardiovascular adjustments, involving a massive increase in total peripheral resistance, were unimpaired by brain transection. In this respect, ducks with RM transections tolerated four-min dives. However, the increase in post-dive $\dot{V}_E$ seen in intact and decerebrated ducks was prevented by RM transection. We conclude that control of the circulatory response to diving resides in the lower brainstem, is reflexogenic in nature, and does not depend on the cognitive perception of 'fearful' stimuli.

Introduction

The cardiovascular responses evoked in reaction to forced diving (apnoea, bradycardia, and peripheral vasoconstriction) are in some respects quite similar to those seen in the fear response which results in freezing [12,40]. Hence, it has been proposed that the two responses are one and the same. Support for this view is engendered by the fact that certain aquatic animals, known to have the characteristic responses to forced submersion, do not show these responses when they are diving voluntarily [25,40]. However, certain observations make it clear that this reasoning is somewhat limited, if not simplistic. There can be no doubt that fear responses must involve cognitive levels of brain arousal for the danger has to be perceived and interpreted as threatening. However, in the laboratory, after first allowing Pekin ducks to breathe pure oxygen in order to deactivate vascular chemoreceptors, the bradycardic response is absent when they are then forcefully immersed [9,11]. Clearly, there is a reflexogenic element in the response to head immersion driven by stimulation of arterial chemoreceptors by the progressively hypoxic and hypercapnic blood which isn't necessarily incorporated into the complex behavioural patterns that comprise the fear response. Furthermore, seals [22,26], muskrats [8], and ducks [4,9,10,42]

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all show some aspects of the characteristic cardiovascular responses to forced submersion even when diving voluntarily.

In a study attempting to resolve the question of the voluntary or reflex nature of the apnoeic response of ducks to submersion, Huxley [16] demonstrated that the apnoeic and bradycardic responses persisted following the destruction of both cerebral hemispheres. Andersen [1] also demonstrated that responses to submersion persisted even after section of the brain below the level of the diencephalon, but owing to the inadequacy of Andersen's description of brain transection, it is not clear if the entire diencephalic region was removed. Certainly, the argument could be raised that some remaining caudal diencephalic centres were still viable and maintaining the diving response. Nevertheless, if a fear response is at all involved in forced diving then even decerebration at the thalamic level might be expected to curtail some aspects of the diving responses in view of the involvement of archistriatal regions in agonistic behaviour in birds [35,38]. There can be no doubt that, as pointed out by Blix and Folkow [3], "decerebrate ducks, ... are fairly difficult to scare."

Thus, the purpose of this investigation was to re-examine the involvement of higher nervous centres in the control of the diving responses. A technique was developed whereby local Xylocaine infusion was used to impose a reversible transection of the brain at the rostral level of the mesencephalon just below the hypothalamic region in decerebrated ducks. Xylocaine blocks both synaptic transmission and conduction along fibres of passage and has been used previously to cause reversible inactivation of discrete areas of brain tissue [29]. Hence, in these experiments on the diencephalic influence on the diving response, decerebrated ducks acted as their own controls for the effects of rostral mesencephalic lesion.

Materials and Methods

Experiments were performed on seven White Pekin ducks weighing between 2.2 and 4.0 kg. Two female and five male ducks were used. Experiments were carried out at room temperature (21–22 °C) and the ducks were acclimatised to this temperature for at least 1 week before experiments.

Decerebration

Decerebration was done under general anaesthesia induced by administration of Halothane (Fluothan, Ayerst Laboratories) into the air stream of unidirectionally ventilated (UDV) animals. 1.8–2.2 vol % Halothane was administered into the inspired air stream (50% oxygen: balance air) by means of a Halothan Vaporiser (Dragerwerk, Lueck, F.R.G.). The brachial artery and vein were cannulated and arterial blood pressure was recorded using a BioTec BT 70 pressure transducer. To augment the hypotensive action of the anaesthetic, a 0.142 μl/ml sodium nitroferricyanide solution (in 5% dextrose) was infused intravenously by means of an infusion pump (Model 901 Harvard Apparatus Co., Millis, MA). Blood pressure was maintained low enough to virtually stop bleeding from the brain tissue as the cerebral hemispheres were extirpated to a level above the thalamus by suction. The operation usually required $1\frac{1}{2}$ h for completion.

The cut edges of the scalp were liberally coated with vaseline and the cranial opening covered with plastic film to prevent dehydration. Several layers of gauze padding were placed over the skull and secured with tape. Each animal was left to recuperate for a minimum of 24 h. Body temperature (Tb) was continously monitored via a rectal thermistor and maintained at 41 ± 1.0 °C with an electric heating pad and/or infra red lamps mounted over the animal.

Reversible rostral mesencephalic (RM) lesion of the brainstem using Xylocaine

Decerebrate ducks were positioned in a stereotaxic apparatus (Narishige, Japan). The dorsal brainstem was exposed and a micropipette injection system was positioned by means of a micromanipulator directly over the caudal end of one optic lobe. The injection system consisted of

a glass micropipette which was connected by polyethylene tubing (P.E. 90, Clay Adams) to a three-way stopcock. One port of the stopcock was connected to a micrometer-driven syringe (AG-LA, Burroughs Wellcome Co., U.K.) which was filled with mineral oil. The other port was connected to a syringe filled with Xylocaine. After the micropipette was back-filled from the Xylocaine syringe, the stopcock was turned to connect the micropipette with the mineral oil in the second syringe. In this way, as the micrometer was turned, the oil/Xylocaine interface could be easily seen moving along the P.E. tubing. Advancing the micrometer by 0.1 mm increments ejected 0.5 μl aliquots of Xylocaine from the pipette tip.

The longitudinal axis of the pipette was tilted at 54° from vertical in alignment with the intended plane of transection passing from the posterior comissure to a point just below the hypophyseal foramen (rostral mesencephalic [RM]). The micropipette was advanced 8 mm into the brain tissue. As it was slowly withdrawn, 0.5 μl Xylocaine was deposited for every 1 mm raised. Once lifted clear of the brain the micropipette was moved 1.5 mm laterally and advanced into the tissue again, with Xylocaine being deposited as before in 0.5 μl quantities for every 1 mm raised. In this manner, Xylocaine was deposited in a planar array across the brainstem. The injection procedure usually took up to 8 min to complete. Tb was continuously monitored and usually began to drop towards the end of the injection period. Areas of the hypothalamus in ducks [33,39] are responsible for thermoregulatory control so that loss of thermoregulation occurs after complete transection across the brainstem below the hypothalamus.

The bird's head was freed from the apparatus and immersed into a beaker of water for diving, but only if a drop in Tb had occurred during the Xylocaine blockade. Care was taken to immerse the head to just above eye level and to ensure that no water spilled into the cranial cavity. Control experiments were carried out using saline injections (0.9% NaCl) and, although Tb did not drop, dives were done at about the same time after saline injection that Tb would have fallen if Xylocaine had been used.

Mechanical RM transection

To accomplish transection between the mesencephalon and the diencephalon, the ducks were re-positioned in the stereotaxic apparatus. A narrow scalpel blade was exchanged for the micropipette in the micromanipulator and the blade drawn through the brain in exactly the same plane as the Xylocaine blockade. Section of tissue down to the ventral side of the brain was completed with a hand-held scalpel.

Experimental protocol

Before decerebration, ducks were placed in a temperature-regulated body-plethysmograph and were subjected to 2-min head submersions in a beaker of cold water (10 °C). Heart rate (HR), blood pressure and Tb were recorded before, during and after submergence. Arterial blood pressure, obtained by connecting the brachial artery cannula to a BioTec BT70 pressure transducer, was recorded on one channel of a 2-channel Techni-Rite TR222 pen recorder (Gulton Inc., RI), writing on rectilinear co-ordinates. Mean arterial pressure (MAP) was obtained from the systolic and diastolic pressures using the formula (Systolic + 2 Diastolic/3). HR was obtained by measuring cardiac intervals from the blood pressure trace. Pre-dive and post-dive ventilation was recorded in 3 ducks. $\dot{V}_E$ was recorded as air-flow in and out of the plethysmograph using a Validyne differential pressure transducer (Model DP103-18, Validyne Engineering Corp., Northridge, CA) connected to a Fleish small animal pneumotachograph. The flow signal was integrated, using a Gould Model 13-4615-70 integrator (Gould Inc., Cleveland, OH) and the integrated signal was displayed on the other channel of the pen recorder. Samples for blood gas analysis were taken before submersion and 10 s before the end of dive from a cannula inserted into the brachial artery. Blood samples were analysed on an IL Model 813 blood gas analyser (Instrumentation Laboratory Inc., Lexington, MA).

The birds were allowed a minimum of 1 day to recuperate from decerebration during which they were regularly force-fed water. Following mechanical brain transection (RM), the animals were allowed at least 6 h to recover during which time

Tb was closely monitored and maintained at 41 ± 1.0 °C by means of electric heating pads and infra red lamps.

The ducks were dived twice while intact, twice after decerebration and twice after mechanical transection (RM) of the brainstem. Each duck was only dived once after reversible rostro-mesencephalic lesion with Xylocaine. In addition to 2-min dives, two of the mechanically RM transected ducks were also subjected to 4-min submergences.

At the end of the experiments, the animals were killed with an overdose of anaesthetic (Sodium Pentobarbital i.v.) and the heads were removed and placed in 5% formal-saline. Two weeks later, the brains were removed and processed for paraffin section. The plane of transection was confirmed by inspection of 12 μm midline sagittal sections stained with Luxol Fast Blue 'G' and Neutral Red.

Statistical analysis

In the text and figures, values are given as means ± SEM. Analysis of variance (ANVART, U.B.C.) was used to test for significant differences within and between ducks when intact, decerebrate, RM lesioned with Xylocaine, and mechanically RM lesioned. Mean values given in the text and figures were established using data from all animals in all dives while statistical analysis only included those animals with a complete data set. Hence, for statistical analysis, N = 7 for heart rate, N = 3 for blood pressure and N = 4 for arterial blood gas values and pH. Since the same ducks were used serially, cardiovascular cannulae were in place for some weeks and loss of patency was the major reason we failed to obtain complete sets of data from all ducks. In order to make heart rate and blood pressure comparisons with the Xylocaine RM lesioned ducks, which were only dived once, the average of the two dives done by the intact, decerebrate and mechanically lesioned RM ducks were used in the analysis of variance. For the ventilatory measurements, values obtained before and after each pair of dives by each animal when intact, decerebrated and mechanically transected were used in the analysis. Where significant F values occurred, Scheffe's test was used to detect significant differences between pairs of means. A probability level of 0.05 was used in establishing a signficant difference.

Results

Reversible RM lesion with Xylocaine

The temporary removal of hypothalamic control following RM Xylocaine blockade was marked by a loss of thermoregulatory function. The fall in Tb reached a maximum approximately 18–20 min from the end of the injection period. The mean fall in Tb for all animals was 1.2 ± 0.2 °C approximately 20 min after brain transection (Fig. 1). Body temperature usually returned gradually to normal between 45 min to 1 h after injection. Control experiments with saline injection never

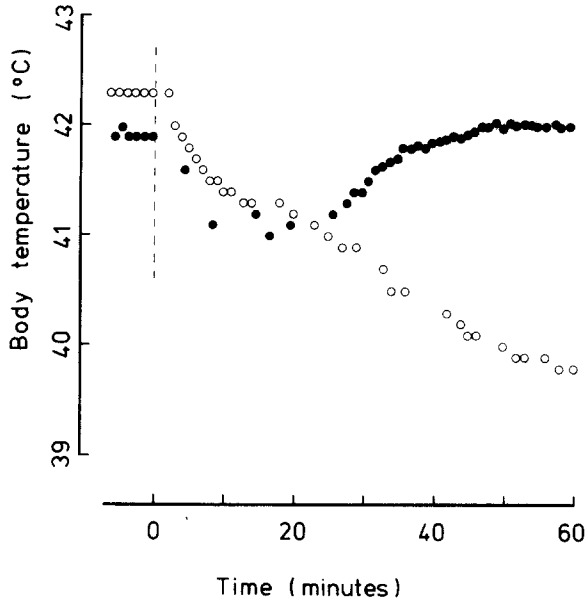

Fig. 1. A typical body temperature profile showing the effect of Xylocaine blockade and brain transection at the rostral mesencephalic (RM) level. Closed circles represent values obtained after infusion of Xylocaine across the rostral mesencephalon. Open circles represent values obtained after mechanical RM transection. Vertical dashed line indicates the time of RM Xylocaine injection and mechanical RM transection.

caused a drop in Tb. In one animal Tb was monitored for 2.5 h after death and it was found that it took 1 h 20 min for Tb to fall 1° C.

Plane of transection

The plane of transection section was identified from the stereotaxic atlas of Zweers [45]. Fig. 2A

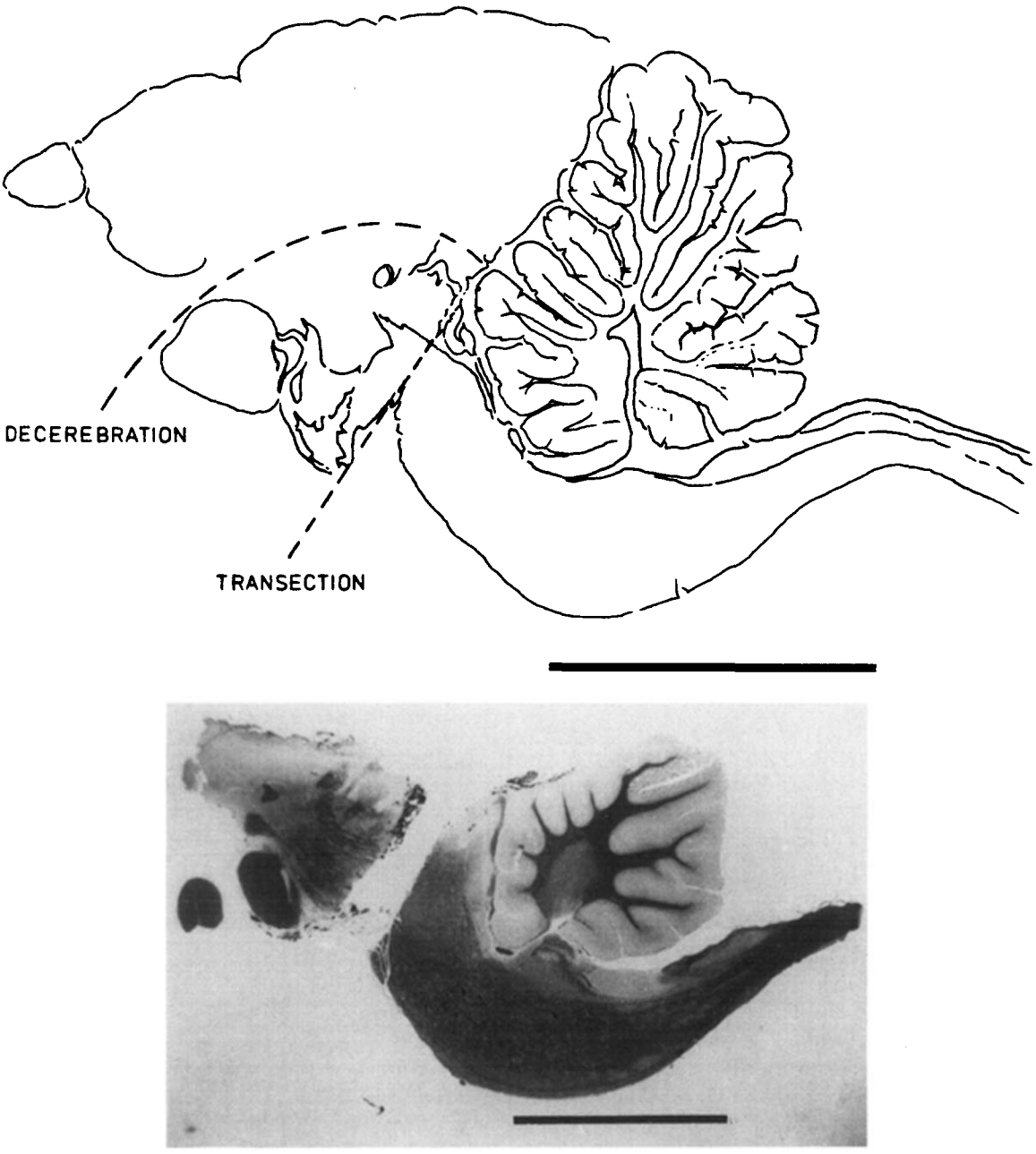

Fig. 2. (**A**) Schematic view of mid-sagittal section of intact brain from the Pekin duck (*Anas platyrhynchos*) indicating level of decerebration and rostral mesencephalic transection (bar = 1 cm). (**B**) Photograph of mid-sagittal section of a decerebrated brain after rostral mesencephalic transection stained with Luxol Blue and Neutral Red (bar = 1 cm).

is a composite diagram of a sagittal view of the brain of a Pekin duck showing the level of decerebration and the plane of section between the mesencephalic and diencephalic regions (RM transection). Fig. 2B is a photograph of a mid-sagittal section from a brain after RM transection.

Diving performance

Resting HR and MAP usually decreased after decerebration and the ducks, though still responsive, were much more passive [35,38]. One day after surgery, they often walked spontaneously and occasionally flexed their wings. Following RM transection, the ducks never exhibited spontaneous activity although they would sometimes stand if encouraged. Differences between resting values in intact, decerebrate and RM transected animals for HR, MAP and blood gases were not significant.

The profile of the fall in HR during submersion was the same in intact and all lesioned ducks. The rate of fall in heart rate over the first 30 s (pre-dive HR minus dive HR at 30 s divided by time (30 s); beats $\cdot$ min^{-1} $\cdot$ sec^{-1}) did not differ significantly between intact and lesioned ducks. Furthermore, the lowest heart rates achieved at the end of the 2-min dives were similar in all ducks, regardless of treatment (Fig. 3). Despite the considerable bradycardia, MAP for all animals was maintained and there were no significant differences between pre- and end-dive values in intact and lesioned ducks. However, in intact ducks, MAP was significantly above end-dive levels early in the dive (10 and 20 s). In intact and lesioned ducks PaO_2 and pHa fell and $PaCO_2$ rose significantly compared with pre-dive levels (Fig. 4). However, as was the case pre-dive, there were no signficant differences between end-dive values of PaO_2, $PaCO_2$ or pHa between intact, decerebrate and RM lesioned ducks.

There was a profound difference in the breathing pattern during recovery from submersion between intact and decerebrate animals when compared with RM transected preparations (Fig. 5). In both intact and decerebrated ducks $\dot{V}_E$ increased significantly from pre-dive levels immediately after surfacing from 2-min dives by about 4

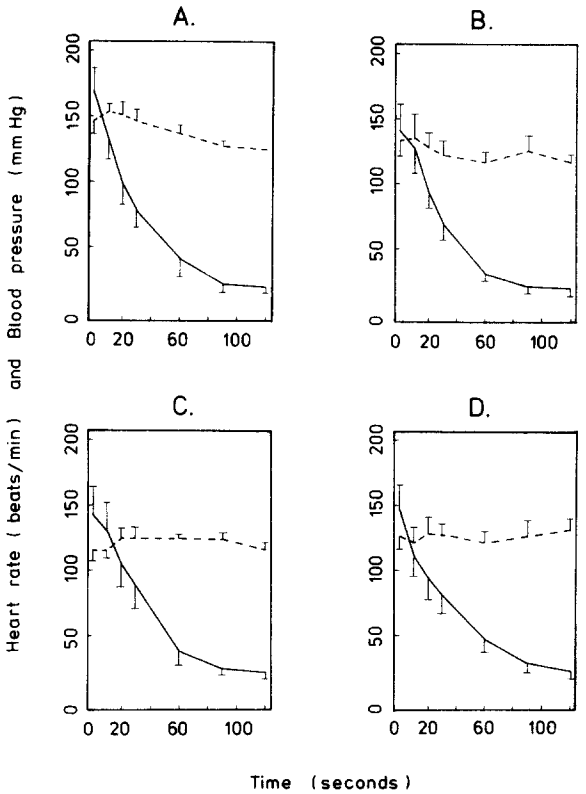

Fig. 3. Effect of various levels of brain transection on the heart rate (solid lines) and mean arterial blood pressure (dashed lines) response to 2 min submergence. (**A**) intact animals (at 120 s the standard error bar on the blood pressure trace is covered by the width of the line); (**B**) decerebrates; (**C**) RM Xylocaine injected animals; (**D**) after mechanical RM mesencephalic transection. For heart rate, Groups A, B and D, N = 7 and n = 14. In group C, N = 6 and n = 6. For mean blood pressure N = 3, n = 6 for Groups A, B and D and N = 3, n = 3 in Group C.

to 5 times, falling gradually to pre-dive levels after about 3 min recovery. However, this was not the case in mesencephalic preparations which never exhibited post-dive hyperventilation. $\dot{V}_E$ in RM lesioned ducks was never significantly above the pre-dive level throughout the recovery period and it was significantly below $\dot{V}_E$ of intact or decerebrated ducks until the end of the recovery period (3 min; Fig. 5). In RM lesioned ducks the pattern of breathing was irregular and sometimes mildly gasping. The RM transected animals survived 4-min submersion periods all the while maintaining bradycardia, but again following

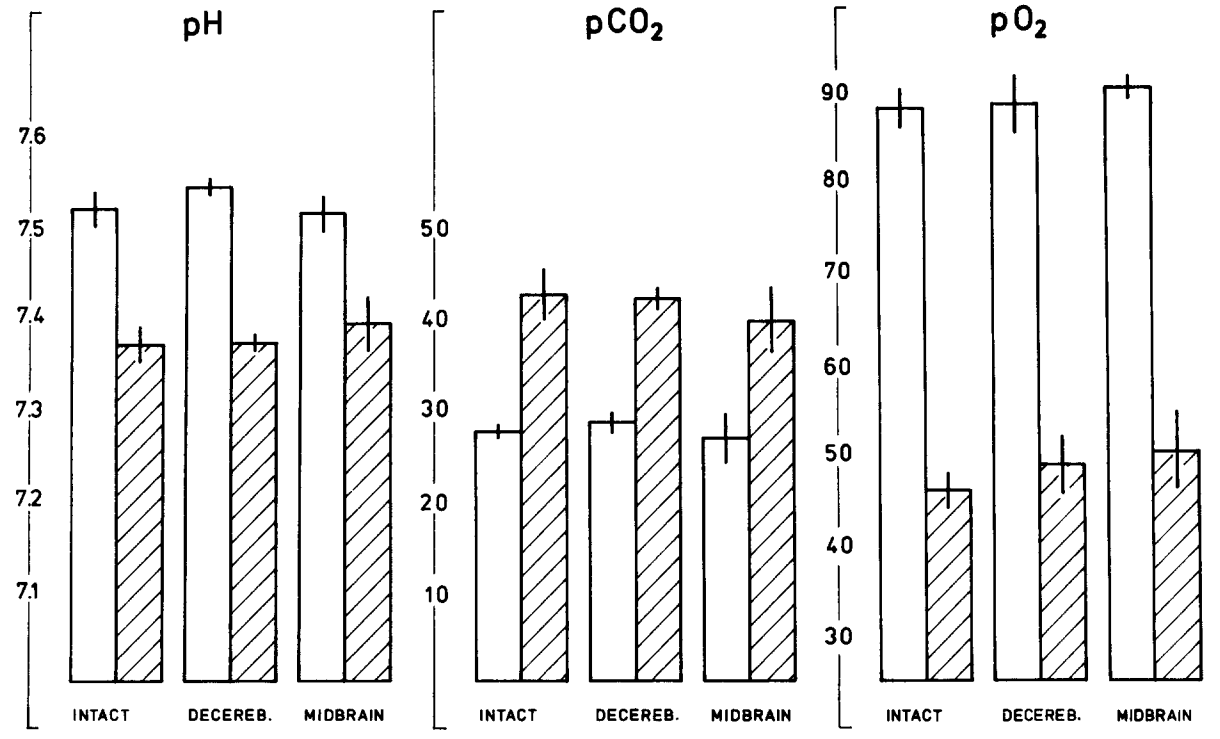

Fig. 4. Effect of brain transection on end-dive blood gas levels and pH. Open bars represent pre-dive values while hatched bars represent values obtained at the end of 2 min submergence. (Intact, N = 7, n = 7; Decerebrate, N = 7, n = 7, RM transection N = 4, n = 4).

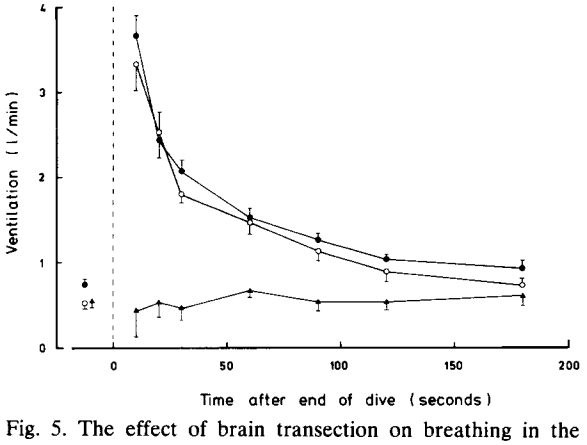

Fig. 5. The effect of brain transection on breathing in the recovery period following 2 min of forced submergence. (●) Intact animals; (○) decerebrate animals; (▲) RM transected animals. The vertical dashed line indicates the end of submersion; the points to the left of this line on each graph represent the predive values for ventilation. (N = 3, n = 6).

these extended dives $\dot{V}_E$ was little different from resting values.

Discussion

The results of this study confirm and extend those of Huxley [16] and Andersen [1]. During dives MAP was maintained at pre-dive levels despite a 70% drop in HR, even in the absence of diencephalic regions (RM transection). Since stroke volume does not increase markedly in dives of 2 to 4 min duration [21], then there must be a compensatory increase in peripheral vascular resistance which serves, as part of the diving response, to limit the loss of stored oxygen to those

tissues (e.g. muscle and gut) undamaged by prolonged periods of oxygen lack. Hence, blood flow is directed towards those tissues, such as the brain and heart, which are crucially dependent on a maintained oxygen supply. The fact that RM transected preparations exhibited cardiovascular adjustments to 2-min submersions that were indistinguishable from intact animals, and also had similar end-dive blood gas values, confirmed that the oxygen conserving response was not lost. Moreover, the ability of these preparations to survive 4-min periods of submersion further demonstrated the effectiveness of the responses. Thus, the control of cardiovascular adjustments and apnoea appears to be localised within the caudal brainstem, below the rostral border of the mesencephalon. Consequently, these results argue against a diencephalon-mediated chemoreceptor-driven bradycardia [27] and they demonstrate that fear (mediated through the telencephalon or diencephalon) is not an essential component of the diving response [25,41].

While it has been assumed that suprabulbar involvement is necessary for multidirectional and differential changes in vascular resistance [20,27, 30,43] the present data in respect to the diving response do not support the concept of complex differential vascular activity under high level CNS control. The predominant feature of vasomotor change during forced submersion is the widespread increase in peripheral vascular resistance. Consequently, the suggestion could be made that, during submersion, sympathetic discharge is increased to vessels throughout the vascular system. That is, the level of sympathetic activation would be close to maximal. Complete vascular shutdown does not occur, however, because of the uneven density of vascular innervation and the presence of adrenergic and cholinergic vasodilatatory fibres. Muscular, mesenteric and renal vessels, for instance, are densely innervated with vasoconstrictor fibres and this is reflected in the greatest decrease of flow in these tissues [20,34,44]. Cerebral, coronary and lung vessels on the other hand are sparsely innervated and blood flow to these tissues has been variously reported as either increasing, remaining the same or decreasing slightly. It also seems likely that auto-regulatory adjustments to hypoxia and hypercapnia in the heart and brain will counter neurally mediated vasoconstriction and increase blood flow. Dilatation during extreme hypoxia has been reported to occur predominantly in coronary and cerebral vessels in contrast to the minimal dilator response of renal and limb vessels [6,13]. Thus, one of the consequences of hypoxic hypercapnia during submersion is almost complete vascular sympathetic activation causing widespread vasoconstriction, excluding a few specific tissues requiring maintained or even increased blood flow. Certainly, high levels of circulating catecholamines occurring during forced submergence [15] imply a global rather than specific role for the autonomic nervous system in promoting increased vascular resistance.

Even so, some degree of differential control mediated by higher nervous centres may exist in intact animals particularly in short dives before circulating catecholamine levels peak. For instance, Djojosugito and co-workers [7] have demonstrated that blood flow in the web of a duck is preserved during submersion primarily through arteriovenous shunts. Maintained cutaneous flow has also been demonstrated in the diving seal [44]. Studies of skin blood flow in the rabbit ear [5] and dog paw [13] revealed that vasodilatation in response to chemoreceptor stimulation by arterial hypoxia was due to differential sympathetic activity [17–19]. A redistribution of blood flow away from skeletal muscle towards the skin can be visualised as part of oxygen-conserving adjustments in view of the abundance of A-V anastomoses in skin and the low rate of oxygen consumption of this tissue. In rabbits, control of this differential activity resides in suprabulbar regions as it is lost following removal of the hypothalamus [19]. It is uncertain, however, what proportion of blood flow is affected by these changes in cutaneous vascular resistance and therefore how much it contributes to oxygen conservation. Much less is known regarding parasympathetic activity, but vagal cardioinhibitory output would appear to be maximal in forced dives [10].

It is well established that chemoreceptor input is a primary instigator of the responses to diving in ducks [23,24]. The reflexes elicited, such as

peripheral vasoconstriction and bradycardia, serve to minimise the cause of the sensory perturbation and, in the case of submersion asphyxia, to conserve oxygen stores. However, it may well be that vascular chemoreceptor input can also activate defence reaction pathways within the brainstem, especially where the efferent output overlaps. It has been shown that vasomotor activity may be evoked by chemoreceptor activation of the defence reaction [2,14,31,32]. In the decerebrated cat with an intact hypothalamus, chemoreceptor stimulation evokes cholinergic vasodilatation in the hind limb along with vasoconstriction in the mesenteric and renal beds [31,32]. It could, therefore, be argued that during submersion chemoreceptor-driven vasconstriction may be attributed to activation of defence reaction output pathways. However, our experiments have shown that the reflex pathways involved in the dive response do not extend above the mesencephalon.

An interesting observation was the loss of post-dive hyperventilation after RM brain transection. However, it is not known how long it took for arterial blood gas levels and pH to return to normal as these were not measured during the recovery period. Although the plane of section corresponded to the rostral border of the mesencephalic region, certain damage may have spread caudally to known respiratory control centres. Areas within the midbrain mediating polypnoea have been identified with respect to thermoregulation in the pigeon [36,37] and stimulation of these sites results in increased respiratory frequency in birds [28]. The loss of the normal post-dive respiratory pattern in mesencephalic animals confirms that important respiratory control resides in regions at or above this level of the brainstem.

This study has shown that control of at least the essential cardiovascular responses to diving are located within the lower brainstem and are not dependent on 'higher centres' of the brain. However, experiments of this kind cannot accurately measure the degree of regional blood redistribution and it may be that in the intact system, higher centres are responsible for 'fine tuning' peripheral vasoconstriction particularly in short dives. Nevertheless, it is clear that responses to diving are reflexogenic in nature and are not dependent on the cognitive perception of 'fearful' stimuli.

Acknowledgements

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