Diving responses in ducks after acute barodenervation

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JONES, DAVID R., W. K. MILSOM, F. M. SMITH, N. H. WEST, AND O. S. BAMFORD. *Diving responses in ducks after acute barodenervation.* Am. J. Physiol. 245 (Regulatory Integrative Comp. Physiol. 14): R222–R229, 1983.—The contribution of systemic arterial baroreceptors to the cardiovascular adjustments to diving has been investigated in unanesthetized ducks after acute baroreceptor denervation. Both intact and denervated ducks exhibited bradycardia on diving, although denervated ducks showed a lesser and more variable fall in heart rate. Hindlimb vascular resistance rose significantly in both intact and denervated ducks. Continuous stimulation of one depressor nerve, through miniature electrodes implanted on the cut central end, resulted in a diving bradycardia intermediate between that recorded in intact and denervated animals. Intermittent stimulation of the depressor nerve, for 20-s periods, at intensities high enough to cause a large fall in mean arterial pressure (MAP) predive caused a smaller reduction in MAP as a dive was prolonged, due to a large decline in the ability of the baroreceptor reflex to affect peripheral resistance. There was no change in the effect of stimulation on cardiac control before or during diving. The present experiments indicate that a barostatic reflex, which exerts its effects primarily through cardiac control and not control of total peripheral resistance, is active through the dive but that the majority of the diving response in ducks is independent of baroreceptor integrity.

arterial baroreceptors; *Anas platyrhynchos*; diving bradycardia; cardiac output; depressor nerve stimulation; arterial blood pressure

DURING FORCED SUBMERGENCE DIVING birds and mammals exhibit apnea accompanied by a group of cardiovascular adjustments that conserve O_2 for the tissues most sensitive to hypoxia, i.e., the myocardium and brain. One such adjustment is a pronounced vasoconstriction of most vascular beds; yet, due to a compensatory decline in cardiac output ($\dot{Q}$), there is little change in mean arterial blood pressure (MAP). This constancy of MAP suggests that systemic arterial baroreceptors might play an important role in these circulatory adjustments (3, 21). In fact, it has even been suggested that diving bradycardia in ducks results from a barostatic reflex activated by an incipient rise in MAP caused by chemoreceptor-induced peripheral vasoconstriction (1, 5). In contrast to this, Jones (11) and Lillo and Jones (19) have shown that chronic denervation of aortic baroreceptors has no significant effect on diving bradycardia. A possible resolution of these discrepant findings was suggested by recent observations indicating that effects of barodenervation differ in acutely (13) and chronically (11, 19) denervated ducks.

Long-term adaptation by the central nervous system in the absence of high-pressure baroreceptors, as in chronic denervates, might mask the true role of barostatic reflexes in the diving response. We therefore set out to look at the effect of acute baroreceptor denervation on MAP, heart rate (HR), and hindlimb vascular resistance (HLVR) before and during diving. In ducks the depressor nerves are branches of the left and right vagi; these branches arise from the lower poles of the nodose ganglia and converge on the aortic root (11). We also implanted stimulating electrodes on the central end of a cut depressor nerve in the denervated ducks (open-loop preparation) and observed the effect of a constant level of depressor nerve activation on the monitored variables. This gave us an indication of the efficacy of the barostatic reflex throughout the dive and postdive recovery period. Furthermore, since it is possible that flow occurring in the hindlimb in dives might be shunted through nonnutritive channels to prevent venous stagnation and facilitate utilization of venous O_2 stores (8), we repeated these experiments measuring $\dot{Q}$ instead of hindlimb flow. This also allowed us to assess the effect of simulated baroreceptor input on total peripheral resistance (TPR) as well as on stroke volume (SV).

METHODS

All experiments were performed on adult ducks (*Anas platyrhynchos*) varying in mass from 1.2 to 3.6 kg. The first group of experiments to be described was performed on Mallard and Khaki Campbell ducks, the second group on White Pekin ducks. This variability was necessitated by the availability of animals but was justified by the uniformity of normal diving responses shown by all three groups of animals. The animals were maintained in closed rooms, under a natural photoperiod, at 20–22°C for at least 1–2 wk before experiments began. All experiments were done at the temperature at which the ducks were maintained. The adjective "normal" describes ducks that had not undergone any operative procedures other than of a superficial nature for exposure of the right cervical vagus, and "sham" describes ducks in

which the baroreceptor reflex could be evoked but which had undergone surgery equivalent to that involved for baroreceptor denervation, although only one depressor nerve was sectioned for implantation of stimulating electrodes. The adjective "predive," when referring to any of the measured variables, describes these before forcible submergence of the head (dive).

Preparatory Surgery

All major operative procedures were done (1–3 days before an experiment) under general anesthesia induced by intramuscular injection of up to 1 $g \cdot kg^{-1}$ of urethan or up to 50 $mg \cdot kg^{-1}$ pentobarbital sodium (Nembutal, Abbott Laboratories, Montreal, Canada). Each duck was restrained, ventral side up, on an operating table, and the feathers covering the area above the clavicular air sac were removed, the skin incised, and the air sac punctured in the midline.

Series I. The depressor nerve, on the left side, was dissected free of surrounding fascia and an associated blood vessel and sectioned close to the brachiocephalic artery. The central end of the nerve was passed through a pair of miniature stimulating electrodes that were then fixed dorsally to surrounding tissue with tissue cement. The stimulating electrodes consisted of two platinum or silver wire (0.2 mm diam) loops of 1.0-mm diameter, embedded in epoxy resin 1 mm apart, and connected to flexible insulated copper wires. The electrode wires were sutured to muscles adjacent to the site where the electrodes were fixed and were also secured to the skin of the neck. This procedure anchored the electrode assembly and ensured the maintenance of good contact between the electrodes and the depressor nerve. The edges of the clavicular air sac were then sewn together until the sac moved with each breath. The skin was then closed over the repaired air sac.

In this group of animals, the right vagus nerve was also exposed by means of a small incision in the skin of the neck. After freeing the nerve from surrounding tissue it was wrapped in rubber dental dam to allow rapid identification on the day of the experiment. The nerve and surrounding tissues displayed no traumatic reaction to the dental dam. At this time, the trachea was also exposed, high in the neck, and one ischiatic artery was exposed in the upper portion of the leg and wrapped in rubber dental dam for later identification. All incisions were then closed. This preliminary exposure of blood vessels and nerves allowed the area of all wounds to heal for 1–3 days before the day of the experiment, since infusions of heparin were required to maintain patency of perfusion cannulas.

All further operative procedures for implantation of cannulas or exposure of nerves, done on the day of an experiment, simply required reopening the closed incisions and were performed under local anesthesia (2% lidocaine). Furthermore the area of any wounds was periodically infiltrated with local anesthetic throughout the course of an experiment.

Arterial blood pressure was recorded from a cannula inserted into either the brachial artery in the wing or ischiatic artery in the leg. The cannula was attached to either a Hewlett-Packard 267BC, Bio-Tec BT 70, or Statham P23Db pressure transducer. A catheter was also inserted into the ulnar vein for drug injections. In this series of experiments the ischiatic artery was used to measure not only arterial blood pressure but also HLVR. The artery was bisected and cannulated both proximally and distally with PE-240 polyethylene tubing, and the two cannulas were forced into the ends of a length of Tygon tubing (20 cm long, 2.4 mm ID, 4 mm OD) that had sidearms close to each end. The tubing, between the sidearms, was passed through the head of a constant-flow peristaltic pump (Watson-Marlow Flow Inducer, Falmouth, Cornwall, UK). Arterial pressure was recorded from the sidearm upstream from the pump head, whereas hindlimb perfusion pressure was recorded from the downstream sidearm. Under control conditions the hindlimb was perfused at a flow rate that yielded the same pressure downstream of the pump as the MAP. The duck was injected with 300–500 $IU \cdot kg^{-1}$ of heparin when hindlimb perfusion was started and about half the initial dose every hour thereafter. Before every experiment the inside of the tubing was treated with a 1% aqueous solution of Siliclad (Clay Adams, Parsippany, NJ), a silicone concentrate. All cannulas and manometers were filled with heparinized avian saline (40 $IU \cdot ml^{-1}$).

For complete baroreceptor denervation in this group the right vagus, reexposed high in the neck, was cooled by placing it on a hollow silver plate through which iced water flowed. On occasion this vagus was sectioned distal to the cooling plate. Cardiovascular and respiratory responses to both unilateral vagal cooling and unilateral vagal section were identical.

Series II. In this group of animals the left depressor nerve was located and sectioned and stimulating electrodes were implanted on the central end of the cut nerve, under general anesthetic, 1–3 days before any experimentation, as described in *series I.* Electromagnetic blood flow probes were also placed around both pulmonary arteries in six animals and around one pulmonary artery in two animals. In the latter animals, a dummy transducer with a lumen diameter similar to that of the recording probe was placed on the other pulmonary artery. The right depressor nerve was exposed between the aorta and right pulmonary artery and sectioned, providing complete baroreceptor denervation. These maneuvers were done through the same cavity, and at the same time, as the placement of the stimulating electrodes.

On the day of an experiment, arterial blood pressure was recorded in these animals from a cannula inserted into a brachial artery. A cannula was also inserted into the ulnar vein for drug injections. Blood flow in the pulmonary arteries was recorded with a Biotronix BL 610 pulsed-logic electromagnetic flowmeter. $\dot{Q}$ was calculated as the sum of the mean flow rate in both pulmonary arteries. When a dummy probe was used on one pulmonary artery the flow recorded in the other pulmonary artery was doubled to give $\dot{Q}$. Zero flow was established during diving when a constant flowmeter output was obtained in diastole. The flowmeter was calibrated at the end of every experiment after the duck was killed

by an overdose of pentobarbital sodium. The artery was cannulated above and below the flow probe, and saline was passed through the vessel from a reservoir. The fluid passing through the transducer was collected in a graduated cylinder and the period for collection of 100 ml timed by stopwatch. We have already established that, for our flowmeter system, saline and blood give identical calibrations when the same probe is tested with both (18).

In both groups of animals, on the day of the experiment, a cannula (4–6 mm OD polyvinyl chloride) was inserted into the trachea and breathing was monitored either with a pneumotachograph attached to the tracheal cannula and the flow signal integrated to give tidal volume, or by measuring the CO_2 and O_2 contents of tracheal gas with a respiratory mass spectrometer (MGA 200, Centronix, Croydon, UK). The mass spectrometer sampled gas at a rate of about 10 $ml \cdot min^{-1}$ and was calibrated periodically with gases of accurately known composition. HR was obtained from an electrocardiogram (ECG) recorded with bipolar copper wire electrodes (6), and after amplification the signal was fed into an instantaneous HR meter to give pulse frequency.

Arterial blood samples, taken from the brachial or ischiatic artery, were analyzed at 41°C with a Radiometer BMS 3 or IL Micro 13 blood gas analyzer with appropriate electrodes. Before analysis the O_2 and CO_2 electrodes were calibrated with precision gas mixtures and the pH electrode with precision buffer solutions.

To judge the effectiveness of denervation, the cardiac chronotropic response was monitored during an elevation in MAP. MAP was increased by intravenous injection of 2 $\mu g \cdot kg^{-1}$ epinephrine through the catheter in the ulnar vein. We have determined that in ducks an intravenous dose of epinephrine sufficient to cause an increase in mean blood pressure of from 20 to 50 mmHg produces no tachycardia or other detectable cardiac effects prior to a baroreflex-induced bradycardia. We have therefore used epinephrine in preference to either phenylephrine or norepinephrine for assessing baroreflex function, since the latter agents induced tachycardia at doses that produced suitable increases in blood pressure.

The rectal temperature of the ducks was monitored in all experiments using a thermistor probe. The body temperature was displayed on a YS1 telethermometer (Yellow Springs Instrument, Yellow Springs, OH), and if body temperature fell below 41°C the bird was warmed by a heating pad or infrared lamp.

All signals were amplified by conventional means, and blood flows and pressure, breathing frequency or integrated tracheal air flow, the O_2 and CO_2 composition of the tracheal air, and the ECG or instantaneous HR were displayed on a Techni-Rite TR888 eight-channel thermal pen writer, writing on rectilinear coordinates. At the same time all variables were recorded on an eight-channel FM tape system for later analysis by computer. The stored data were analyzed using a specially prepared computer program for a Digital PDP Lab 8e computer. This program yielded the mean values of the analog data, e.g., blood pressures and HR, over preset but variable time periods.

Experimental Protocol

In all experiments the animals were restrained in a prone position on an operating table, with the head held pointing downward into a large filter funnel. Head submergence was effected by filling the funnel from a beaker of water after first clamping a piece of tubing attached to the spout. Removal of the clamp drained the funnel for emersion.

Series I. Effects of acute baroreceptor denervation and of stimulation of the depressor nerve on blood pressure, heart rate, and hindlimb vascular resistance. In this series of experiments we attempted not only to show the effects of acute denervation on the diving response, compared with sham controls, but also to standardize the denervation procedure. Experiments were done on six Mallard and Khaki Campbell ducks (mean mass 1.77 ± 0.12 kg) in which acute baroreceptor denervation was effected during the course of the experiment. In ducks the baroreceptors are located at the root of the aorta, innervated by a branch (exceptionally, branches) of the vagus nerve (11). The left vagal branch was sectioned for implantation of stimulating electrodes, so cooling or section of the right vagus gave a baroreceptor-denervated animal. Hence it was possible to reversibly denervate the duck (by cooling) so that each animal acted as its own control. It was necessary, however, to check the effect of unilateral vagotomy per se on the diving response. This was done in experiments on three Mallard ducks with an average mass of 1.80 ± 0.13 (SE) kg. HR was recorded before, during, and after forced submergence of 2-min duration. On the 1st day of experimentation, three normal dives were alternated with dives when the right vagus was cooled. After these dives the right vagus was cooled, injected with local anesthetic distal to the cooling plate, and sectioned. The duck was dived again after unilateral vagotomy.

Stimulation of the left depressor nerve was done only in animals denervated by unilateral cooling or section of the right vagus nerve. The stimulating electrodes were connected to a Grass S4 stimulator (Grass Instruments, Quincy, MA), and the nerve was stimulated using the same stimulus parameters before, during, and after dives of 2-min duration. In contrast to experiments reported in *series II*, the stimulus parameters were set to yield approximately the same HR and MAP in denervates as existed in the animals before denervation in the predive state. Stimulation affected only cardiovascular variables. There were no motor or other behavioral effects associated with the period of stimulation that might be associated with the delivery of a painful stimulus. In these experiments the hindlimb was perfused, at constant pressure, and flow to the hindlimb was recorded. We chose constant-pressure rather than constant-flow perfusion, since the former gave values for HLVR in the same range as those obtained from autoperfused hindlimbs (7). The pump used to perfuse the hindlimb was calibrated over the range of diving pressures (MAP) encountered in an experiment, at the termination of the experiment. In cases where a change in diving pressure affected flow the recorded flow values were adjusted accordingly. HLVR

was expressed as the quotient of perfusion pressure (mmHg) and blood flow (ml·min^{-1}). Central venous pressure was ignored in this calculation, since, although it increases markedly in a dive (11), it was always less than 10% of the perfusion pressure in the experiments reported here.

Series II. Effects of stimulation of the depressor nerve on blood pressure, cardiac output, and total peripheral resistance. These experiments were done on eight White Pekin ducks (mean mass 2.78 ± 0.13 kg) in which both depressor nerves were sectioned at least 1 day before the experiments. Consequently no recordings were made from shams with intact barostatic reflexes. Nevertheless we felt that these experiments would yield enough data on the major cardiovascular variables for a complete analysis of the effects of acute denervation. Furthermore the left depressor nerve was stimulated using voltage-frequency parameters which gave a marked fall in blood pressure before submergence, because we wished to accentuate the changes in these variables, particularly during the later stages of the dive. Stimulation was maintained from before to 2 min after a dive, or intermittent (20-s duration) periods of stimulation were given before, during, and after submergence. During intermittent stimulation all variables were measured 10–15 s after the onset of stimulation. MAP, $\dot{Q}$, and HR were measured, and TPR was calculated as the quotient of MAP and $\dot{Q}$. No allowance was made for the rise in venous pressure in diving when making this calculation.

Calculations and Statistical Analysis of Data

In the text and tables numerical values, when referring to determinations of variables in a group of animals, are given as means ± SE of n observations on N 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 (23)]. Comparisons of data within a group were conducted using a two-factor analysis with repeated measures over time (UBC, ANOVAR). In the case of significant F values ($P < 0.05$), pair-wise comparisons of means were done with either Scheffé's method (24) or the LSD test (25).

RESULTS

Series I: Effect of Stimulation of the Depressor Nerve on Mean Arterial Pressure, Heart Rate, and Hindlimb Vascular Resistance During Diving

Preliminary experiments: effect of unilateral vagotomy on diving bradycardia. Mean surface HR of normal Mallards (179 ± 21 beats·min^{-1}, $n = 9$, $N = 3$) was not significantly different from that obtained from ducks with a cooled or sectioned right vagus nerve (158 ± 11 beats·min^{-1}, $n = 16$, $N = 3$). There was no difference in either resting HR or development of bradycardia in animals unilaterally vagotomized by vagal cooling or sectioning, so data from these two groups were pooled in data analysis. In both normal and unilaterally vagotomized ducks the fall in HR in the early part of a 2-min dive was similar. After 1 min diving, normal animals had an average rate of 25 ± 6 vs. 32 ± 5 beats·min^{-1} in unilaterally vagotomized ducks. HR continued to decline and, after 2 min diving, attained 10% of the surface rate in normal ducks. This rate, 18 ± 4 beats·min^{-1}, was significantly below that in unilaterally vagotomized animals (29 ± 3 beats·min^{-1}) in which HR fell to only 18% of the predive rate. In absolute terms unilateral vagotomy had only a slight effect on diving bradycardia; however, on a proportionate basis this difference was magnified; i.e., in normal ducks during a dive HR fell to one-tenth predive values, whereas in unilaterally vagotomized ducks, the HR fell to only one-fifth the predive HR.

Stimulation experiments. We have divided the animals in these series of experiments into three groups: *1*) sham, in which one depressor nerve was intact; *2*) denervated, unilaterally vagotomized animals with no baroreceptor innervation or stimulation; and *3*) stimulated, in which a depressor nerve was continuously stimulated in a unilaterally vagotomized, baroreceptor denervate (Table 1). In all three groups MAP rose during diving. This increase was not significant compared with predive values in intact animals, although it was in stimulated animals after both 1 and 2 min submergence (Table 1). In stimulated ducks the rise in MAP was about 43 mmHg after 2 min diving. On average the fall in HR in a dive was least in intact animals (246 beats·min^{-1}) and greatest in denervates (270 beats·min^{-1}), but since denervation caused resting HR to increase significantly by 123 beats·min^{-1}, the proportionate reduction in HR in denervates (to 35% of predive) was only half that in intact animals (to 18% of predive). It must be pointed out, however, that not all of the reduction in the degree of bradycardia can be attributed to baroreceptor denervation, since this procedure was accompanied by unilateral vagotomy,

TABLE 1. *Changes in some cardiovascular variables during dives of 2-min duration*

	Sham	Denervated	Stimulated
		Predive	
MAP, mmHg	143.3±10.0	202.2±20.5*S	131.8±13.1*D
HR, beats·min^{-1}	299.3±29.0	422.5±17.3*S	365.3±33.0
HLVR, PRUs	5.8±0.9	7.3±1.1	4.8±1.4
		1-Min dive	
MAP, mmHg	161.2±8.9	245.2±20.0† *S	182.8±16.4†
HR, beats·min^{-1}	81.3±20.0†	208.5±25.6† *S	132.8±5.3†
HLVR, PRUs	22.7±1.0†	26.0±3.9†	19.1±6.0†
		2-Min dive	
MAP, mmHg	162.5±6.2	226.0±25.7	174.3±16.2†
HR, beats·min^{-1}	53.0±3.9†	152.5±15.8† *S	102.5±10.5† *S *D
HLVR, PRUs	48.4±17.2†	37.5±9.1†	30.5±2.5†

Means are of 6 observations on 6 sham and 6 denervated ducks, during 2-min dives and continuous stimulation of central end of cut depressor nerve in denervated ducks. MAP, mean arterial pressure; HR, heart rate; HLVR, hindlimb vascular resistance. * Significant difference from S (sham) and/or D (denervated). † Significant difference from predive value.

TABLE 2. *Changes in some cardiovascular variables during dives of 2-min duration*

	Predive		Dive					
			0.5 min		1 min		1.5 min	
	Den	Stim	Den	Stim	Den	Stim	Den	Stim
MAP, mmHg	136.8* ±8.0	53.5 ±4.1	212.5*† ±12.5	106.6† ±10.1	141.8* ±10.8	81.8† ±8.7	133.0* ±10.5	77.1† ±4.9
HR, beats·min^{-1}	294.4 ±15.8	261.7 ±16.0	130.0† ±17.7	165.0† ±22.0	98.8† ±14.5	74.2† ±9.6	90.6† ±13.5	81.8† ±17.9
$\dot{Q}$, ml·min^{-1}	664.4 ±63.8	516.6 ±69.3	279.6† ±50.4	302.5† ±55.0	141.4† ±24.3	126.3† ±24.6	156.7† ±39.5	129.6† ±21.7
TPR, PRU	0.223* ±0.026	0.122 ±0.017	0.783*† ±0.090	0.356† ±0.052	1.472† ±0.254	1.100† ±0.314	2.052† ±0.485	0.810† ±0.347

	Recovery									
	0.15 min		0.5 min		1 min		1.5 min		2 min	
	Den	Stim	Den	Stim	Den	Stim	Den	Stim	Den	Stim
MAP, mmHg	164.0 ±10.5	134.4† ±10.8	146.8* ±12.6	102.1† ±13.4	153.8* ±11.1	93.7† ±9.1	210.8*† ±16.9	138.9† ±14.4	168.9* ±14.6	89.0† ±9.7
HR, beats·min^{-1}	362.5 ±15.5	380.8 ±29.4	413.1† ±12.7	388.3 ±21.1	383.8† ±15.4	335.8 ±12.2	373.7*† ±16.1	297.0 ±15.9	371.0*† ±14.4	256.0 ±11.5
$\dot{Q}$, ml·min^{-1}	802.1 ±101.0	801.8 ±81.0	1,155.4† ±97.8	1,018.4 ±98.0	1,090.8† ±84.3	914.1 ±87.4	999.6† ±84.4	818.1 ±100.6	872.6 ±96.6	785.4 ±66.2
TPR, PRU	0.256 ±0.029	0.189 ±0.022	0.153 ±0.021	0.108 ±0.012	0.161 ±0.020	0.114 ±0.014	0.201 ±0.038	0.141 ±0.019	0.237 ±0.044	0.158 ±0.034

Means are of 8 observations on 8 denervated ducks (Den) before and during 1.5-min dives when the central end of cut depressor nerve was continuously stimulated (Stim). MAP, mean arterial pressure; HR, heart rate; $\dot{Q}$, cardiac output; TPR, total peripheral resistance. * Significant difference between values from denervated and stimulated animals at a given time. † Significant difference from the respective predive values.

TABLE 3. *Fall in BP, mean pressure during stimulation, and proportionate changes in other cardiovascular variables*

	Predive	Dive		Recovery	
		1 min	2 min	1 min	2 min
Fall in BP, mmHg	91.8 ±6.8 (16)	81.4* ±9.0 (16)	46.2† ±8.1 (12)	49.7*† ±6.4 (10)	84.1 ±6.7 (16)
Pressure during stimulation, mmHg	75.5 ±7.1 (16)	126.7† ±10.2 (16)	124.7† ±10.0 (12)	136.5† ±19.9 (10)	118.4† ±12.9 (16)
Proportionate MAP, %	54.5 ±2.7 (12)	36.7† ±3.3 (12)	27.0† ±3.9 (12)	29.8*† ±5.0 (10)	47.2 ±4.9 (10)
Proportionate $\dot{Q}$, %	34.2 ±4.9 (10)	40.0 ±8.1 (10)	24.0 ±8.3 (10)	19.0 ±4.2 (10)	17.4 ±5.3 (10)
Proportionate SV, %	9.7 ±4.7 (16)	−17.2 ±24.7 (15)	−14.6 ±17.4 (10)	−8.0*† ±5.0 (9)	14.3 ±5.1 (15)
Proportionate HR, %	29.0 ±3.5 (11)	49.2* ±5.3 (11)	18.6 ±11.1 (11)	24.5 ±3.5 (9)	31.9 ±2.0 (9)
Proportionate TPR, %	22.3 ±5.4 (10)	−30.3† ±20.4 (10)	−10.1† ±12.1 (10)	13.0* ±3.6 (10)	33.3 ±4.5 (10)

No. in brackets under each mean is no. of observations on 8 ducks during intermittent periods of stimulation of depressor nerve before, during, and after dives of 2.5- to 3.5-min duration by denervated ducks. Proportionate change was calculated as [1 − (Vs/Vp)] × 100, where Vs is value of variable during stimulation and Vp is value of variable immediately before stimulation. BP, blood pressure; MAP, mean arterial pressure; $\dot{Q}$, cardiac output; SV, stroke volume; HR, heart rate; TPR, total peripheral resistance. * Significant difference between 1- and 2-min values in dive and in recovery. † Significantly different from predive value.

which also reduces the extent of bradycardia (see above). Stimulated animals were intermediate in both absolute and proportionate reductions in HR (Table 1). HLVR increased in a similar fashion in all three groups of ducks, and at 2 min submergence these values were not significantly different from one another (Table 1). On a proportionate basis, HLVR increased about five to eight times. Arterial blood gas and pH values were similar in all three groups both before and after 1.5 min diving.

Series II. Effect of Stimulation of the Depressor Nerve on Blood Pressure, Cardiac Output, and Total Peripheral Resistance During Diving

In these experiments, dives with continuous (Table 2) or intermittent (Table 3) stimulation were compared with dives by denervated animals. In denervated animals, HR fell to 30% of the predive value after 1.5 min submergence, whereas $\dot{Q}$ declined to less than one-quarter, indicating a fall in SV. MAP initially rose significantly (0.5 min dive) but then declined to predive levels. TPR increased significantly by 9.2 times (Table 2). These changes were reversed when the animal resumed breathing after emergence. After 0.5 min breathing, $\dot{Q}$ and HR were significantly above predive values and HR remained so for the rest of the 2-min recovery period. TPR returned to the predive level within 0.15 min of surfacing.

Despite continuous stimulation of the depressor nerve, MAP rose significantly during diving. After 1 min underwater MAP had risen significantly by 28 mmHg and remained elevated for the rest of the dive and recovery period (Table 2). $\dot{Q}$ was not significantly different from

that in denervates at any time during the dive. TPR was significantly below that in denervates after 0.5 min underwater; however, this significant difference was not maintained for the rest of the dive. Continuous stimulation during the first 0.5 min of the recovery period had little effect on any of the cardiovascular variables, and blood pressure was at its highest at this time. MAP remained significantly above predive values throughout the recovery period. $\dot{Q}$ and HR were also significantly above the predive values early in recovery and were similar to corresponding values in denervates. As recovery progressed, however, HR returned more rapidly to predive levels in stimulated animals than in denervates. TPR fell rapidly after emergence and was not significantly different from predive after 0.15 min.

Intermittent stimulation of the depressor nerve (for 20-s periods) 0.5 min before diving caused significant reductions in all measured cardiovascular variables (Table 3). MAP fell, as a proportion of MAP immediately before stimulation, by 55 ± 3%. $\dot{Q}$ fell, as a proportion of prestimulation $\dot{Q}$, by 34 ± 5%, which was almost totally due to HR falling proportionately by 29 ± 3% (Table 3). In other words, SV was unaffected by stimulation. TPR fell, as a proportion of prestimulation TPR, by 22 ± 5%. During diving the amount by which MAP fell during stimulation, as a proportion of the immediate prestimulation value, decreased significantly. After 1 min MAP fell proportionately by 37 ± 3% and at 2 min by only 27 ± 4% (Table 3). At no time in the dive was the proportionate reduction in $\dot{Q}$ or HR significantly different from the predive value, so this loss in effectiveness of depressor nerve stimulation on MAP was due to a difference in the ability of nerve stimulation to alter TPR. In fact, after 1 min submergence, TPR increased during stimulation so, on a proportionate basis, the change was negative (−30 ± 20%; Table 2). This difference in the proportionate effect on TPR before and during diving was significant. Stimulation 1 min into the recovery period reduced MAP by the same proportionate amount as occurred at the end of the dive. The proportionate effects on $\dot{Q}$, TPR, and HR were also not significantly different from those at end-dive. After 2 min recovery, MAP was reduced by the same proportionate amount, on depressor nerve stimulation, as occurred predive. The greater effectiveness of stimulation in this period was due to the fact that, proportionately, TPR was now reduced by 33 ± 4% (Table 3).

DISCUSSION

From the present experiments, it is obvious that a substantial portion of the cardiovascular response to forced submergence in ducks occurs in the absence of an intact barostatic reflex. This reflex does play a role in the diving response; however, that role is relatively small. This conclusion conflicts with the opinion of Andersen and Blix (1) who suggested that the profound bradycardia in diving ducks is due to the barostatic reflex operating in response to peripheral vasoconstriction. This suggestion was based on experiments in which epinephrine was injected into two reserpinized ducks, early in dives, causing an elevation in blood pressure and bradycardia. In our opinion these experiments show only that the barostatic reflex operates during diving, which is confirmed by the present work.

Part of the controversy surrounding the role of baroreceptors in diving must stem from emphasizing the relation between various components of the effector arm of the barostatic reflex (such as HR or HLVR) and MAP, rather than the role of baroreceptors in controlling MAP per se. For instance, Angell James, Daly, and Elsner (3) claim that in diving seals the relation between cardiac interval and MAP is reset toward bradycardia. These observations in the seal complemented previous suggestions that resetting also involved the vasomotor-pressure relationship (2). Changes in the relation between heart period and blood pressure, however, can have both baroreceptor-dependent and -independent components. The trigeminal input, increased chemoreceptor drive, and cessation of rhythmic activity in both central respiratory neurons and pulmonary afferents during diving result in a reduction in HR, whereas MAP remains constant, altering the relation between HR and blood pressure. Much of this alteration appears to be baroreceptor independent (17). These baroreceptor-independent changes, when ignored, lead to an overestimation of the importance of baroreceptors in cardiac and vasomotor control in dives. In the present experiments we examined the effect of baroreceptor stimulation in causing changes in MAP, eliminating this source of error.

The present experiments show that assessments of the role played by baroreceptors in generating cardiovascular responses to diving will differ depending on whether studies are made in acute or chronic baroreceptor denervates. Acute baroreceptor denervation reduces the degree of diving bradycardia, an effect that can be offset to some extent by continuous central stimulation of the cut depressor nerve (Tables 1 and 2). Also intermittent depressor nerve stimulation affects HR before a dive in the same way that it does after 2 min submergence, whereas TPR falls during predive stimulation but generally rises in response to in-dive stimulation (Table 3). Consequently, from these experiments, we would conclude that the barostatic reflex in ducks exerts its effects primarily through cardiac control and not control of TPR. Work with chronic denervates, however, leads to the opposite conclusion (11, 19). In chronic denervates, blood pressure falls markedly in dives due to the failure of peripheral resistance to increase, whereas HR falls to the same level as in intact ducks.

During dives, MAP rises in acute denervates and falls in chronic denervates, so it is reasonable to conclude that baroreceptors play a role in maintaining the blood pressure of intact ducks during dives. It is the manner in which this role is carried out that is a matter of dispute. Certainly large changes in TPR occur that are totally independent of baroreceptor activation in both chronic (11) and acute denervates, so in intact animals the absolute effects of the barostatic reflex on cardiac rate and output need only be relatively slight to achieve homeostatic regulation of blood pressure. The fact that large baroreceptor-independent changes in TPR occurred in dives in the present and in previous work in ducks (13) strongly suggests that bradycardia and the increase in

TPR are not necessarily inexorably linked, a conclusion reached earlier by Murdaugh and co-workers (22) in the seal.

Experiments with intermittent stimulation of the depressor nerve showed that the properties of the barostatic control system are altered during diving. Stimulation of the depressor nerve during diving had significantly less effect on MAP as the dive progressed. Depressor nerve stimulation had virtually the same effect on HR and $\dot{Q}$ before and during dives, but the effect on TPR was reduced or even reversed compared with predive; stimulation in the dive caused an increase in peripheral resistance. This alteration (or resetting) of the properties of the barostatic control system in ducks has similarities to that occurring in mammals during chemoreceptor activation (4, 9, 10, 14–16, 20, 26). Consequently, it is tempting to suggest that in dives the chemoreceptor input is all important for modulating the barostatic control system. However, after the 1st min of recovery from diving, when blood gas levels (hence chemoreceptor input) have returned to predive levels, depressor nerve stimulation gave the same proportional reduction in MAP as did stimulation at the end of the dive.

An important point that needs to be addressed is how much of the apparent effect of acute baroreceptor denervation is caused by the increased stress of the operative techniques. Unfortunately this point was not directly investigated in our experiments. In the experiments where comparison was made between sham- and acutely baroreceptor-denervated ducks (*series I*), baroreceptor denervation involved unilateral vagotomy and not solely section of the remaining depressor nerve. Unilateral vagotomy alone had no effect on resting HR or on bradycardia until the 2nd min of submergence, when bradycardia was not as profound as in normal ducks. In terms of absolute numbers the effect was small, the difference being 10–15 beats·min^{-1}, but proportionately this diving HR represented double the HR attained during diving in normal ducks. Section of one depressor nerve (sham), rather than the entire vagus, had a marked effect on resting HR, and in dives HR was almost three times that in normal ducks. However, due to the marked elevation in resting HR, the proportional drop in HR (to 17% of the predive value) was similar to that found in unilaterally vagotomized animals (to 18% of predive HR). It is difficult to say why animals with unilateral depressor nerve section should have much higher resting HR than unilaterally vagotomized animals other than the fact that these animals had undergone significantly more surgical intervention in the days preceding the experiments as well as on the day of the experiment. If the effects of unilateral vagotomy and the sham operation were additive, we would expect diving HR in unilaterally vagotomized ducks (denervated of Table 1) to be four to five times higher than that in normal ducks. However, in unilaterally vagotomized acute baroreceptor denervates, diving HR was about eight times higher than that in normal ducks (Table 1), suggesting that acute baroreceptor denervation per se also doubles diving HR. This line of argument is supported by the fact that in stimulated animals (Table 1) where the effects of acute barodenervation are reversed, but not the effect of the unilateral vagotomy or sham operation, HR was four to five times that found in normal ducks. We therefore acknowledge a series of confounding factors in these experiments. Nonetheless, we feel that they do not alter our basic interpretation of the data. The majority of the diving response in ducks is due to the stimulation of peripheral and central chemoreceptors by the progressively more hypoxic and hypercapnic blood (12, 13), a process independent of baroreceptor integrity (13).

A minor point resolved by the present experiments is that hindlimb peripheral resistance gives a reasonable indication of the change in TPR. The fact that changes in TPR (6–10 times) were somewhat larger than changes in HLVR (5–8 times), under similar conditions, could indicate that some flow may by-pass the nutritive vascular beds in the leg through arteriovenous shunts as suggested by Djojosugito et al. (8).

The major point of the present work, however, remains unresolved. The reason for the difference between acute and chronic denervates in cardiovascular performance during dives, aside from effects of surgery, is unknown. We feel that this difference is real and must lie in some long-term property of adaptation by the central nervous system to functioning without high-pressure baroreceptors.

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REFERENCES

1. ANDERSEN, H. T., AND A. S. BLIX. Pharmacological exposure of components in the autonomic control of the diving reflex. *Acta Physiol. Scand.* 90: 381–386, 1974.
2. ANGELL JAMES, J. E., AND M. DE B. DALY. Some mechanisms involved in the cardiovascular adaptations to diving. *Symp. Soc. Exp. Biol.* 26: 313–341, 1972.
3. ANGELL JAMES, J. E., M. DE B. DALY, AND R. ELSNER. Arterial baroreceptor reflexes in the seal and their modification during experimental dives. *Am. J. Physiol.* 234 (*Heart Circ. Physiol.* 3): H730–H739, 1978.
4. ATTINGER, F. M. L., E. O. ATTINGER, D. COOPERSON, AND W. GOTTSCHALK. Interactions between carotid sinus mechanoreceptor and chemoreceptor reflex loops. *Pfluegers Arch.* 363: 255–261, 1976.
5. BLIX, A. S., E. L. GAUTVIK, AND H. REFSUM. 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.
6. BUTLER, P. J., AND D. R. JONES. Onset of and recovery from diving bradycardia in ducks. *J. Physiol. London* 196: 255–272, 1968.
7. BUTLER, P. J., AND D. R. JONES. 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, 1971.
8. DJOJOSUGITO, A. M., B. FOLKOW, AND L. R. YONCE. Neurogenic adjustments of muscle blood flow, cutaneous A-V shunt flow and

of venous tone during "diving" in ducks. *Acta Physiol. Scand.* 75: 377–386, 1969.
9. HEISTAD, D. D., F. M. ABBOUD, A. L. MARK, AND P. G. SCHMID. Interaction of baroreceptor and chemoreceptor reflexes. Modulation of the chemoreceptor reflex by changes in baroreceptor activity. *J. Clin. Invest.* 53: 1226–1236, 1974.
10. IRIKI, M., P. DORWARD, AND P. I. KORNER. Baroreflex "resetting" by arterial hypoxia in the renal and cardiac sympathetic nerves of the rabbit. *Pfluegers Arch.* 370: 1–7, 1977.
11. JONES, D. R. Systemic arterial baroreceptors in ducks and the consequences of their denervation on some cardiovascular responses to diving. *J. Physiol. London* 234: 499–518, 1973.
12. JONES, D. R., AND M. J. PURVES. The carotid body in the duck and the consequences of its denervation upon the cardiac responses to immersion. *J. Physiol. London* 211: 279–294, 1970.
13. JONES, D. R., W. K. MILSOM, AND G. R. J. GABBOTT. Role of central and peripheral chemoreceptors in diving responses of ducks. *Am. J. Physiol.* 243 (*Regulatory Integrative Comp. Physiol.* 12): R537–R545, 1982.
14. KIRCHHEIM, H. R. Systemic arterial baroreceptor reflexes. *Physiol. Rev.* 56: 100–176, 1976.
15. KORNER, P. I. Integrative neural cardiovascular control. *Physiol. Rev.* 51: 312–367, 1971.
16. KORNER, P. I. Circulatory homeostasis—role of reflex interactions. *Proc. Aust. Physiol. Pharmacol. Soc.* 10: 102–111, 1979.
17. KORNER, P. I., J. SHAW, M. J. WEST, J. R. OLIVER, AND R. G. HILDER. Integrative reflex control of heart rate in the rabbit during hypoxia and hyperventilation. *Circ. Res.* 33: 63–73, 1973.
18. LANGILLE, B. L., AND D. R. JONES. Central cardiovascular dynamics of ducks. *Am. J. Physiol.* 228: 1856–1861, 1975.
19. LILLO, R. S., AND D. R. JONES. Control of diving responses by carotid bodies and baroreceptors in ducks. *Am. J. Physiol.* 242 (*Regulatory Integrative Comp. Physiol.* 11): R105–R108, 1982.
20. MANCIA, G. Influence of carotid baroreceptors on vascular responses to carotid chemoreceptor stimulation in the dog. *Circ. Res.* 36: 270–276, 1975.
21. MILLARD, R. W. Depressed baroreceptor-cardiac reflex sensitivity during simulated diving in ducks. *Comp. Biochem. Physiol. A* 65: 247–249, 1980.
22. MURDAUGH, H. V., JR., C. E. CROSS, J. E. MILLEN, J. B. L. GEE, AND E. D. ROBIN. Dissociation of bradycardia and arterial constriction during diving in the seal *Phoca vitulina*. *Science* 162: 364–365, 1968.
23. NIE, N. M., C. M. HALL, J. G. JENKINS, K. STEINBRENNER, AND D. BENT. *SPSS, Statistical Package for the Social Sciences.* New York: McGraw-Hill, 1975.
24. SCHEFFÉ, H. *The Analysis of Variance.* New York: Wiley, 1959.
25. SNEDECOR, G. W., AND W. G. COCHRAN. *Statistical Methods* (6th ed.). Ames, IA: Iowa State Univ. Press, 1967.
26. WENNERGREN, G., R. LITTLE, AND B. ÖBERG. Studies on the central integration of excitatory chemoreceptor influences and inhibitory baroreceptor and cardiac receptor influences. *Acta Physiol. Scand.* 96: 1–18, 1976.