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The Source of Circulating Catecholamines in Forced Dived Ducks

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Plasma catecholamines have been measured in chronically adrenalectomized (ADX) ducks, in chronically adrenal-denervated ducks (DNX), and in their respective sham-operated controls (SH-adx, SH-dnx) after 3 min forced submergence. The results showed that 100% of the plasma epinephrine (EP) and 70 to 80% of plasma norepinephrine (NE) released during the dive came from the adrenal glands. Only 20 to 30% of plasma NE came from the endings of the autonomic vascular sympathetic nerves which are strongly stimulated during diving. Adrenal catecholamines were released by nerve activation only; nonneural mechanisms did not play any role in their release. The action of adrenal catecholamines on the cardiovascular system during dives was investigated by measuring heart rate and arterial blood pressure in operated and sham-operated ducks. Cardiovascular adjustments, associated with 3 min of forced diving, were not affected by any differences in the levels of plasma catecholamines.

Diving animals show a remarkable increase in plasma free catecholamines (norepinephrine, NE; epinephrine, EP) during forced submergence. In the duck, plasma catecholamine concentrations double after a dive of 1 min (Huang *et al.*, 1974) and increase 1000-fold after 10 to 14 min submergence (Hudson and Jones, 1982). Tenfold increases in catecholamines have also been observed in the Harbor seal after 4 to 6 min diving (Hance *et al.*, 1982). Mangalam *et al.* (1987) showed that catecholamine release was triggered mostly by hypoxia during diving. Acidosis, hypercapnia, and even a stress component related to the handling of the animal for the dive itself had little affect on catecholamine release. By comparing adrenal-denervated ducks with sham-operated ducks, Mangalam *et al.* (1987) showed that half of the plasma NE and 90% of plasma EP released during dives came from neural activation of the adrenal glands. They thought that nonneural releasing mechanisms could also have triggered catecholamine release from the adrenals during forced dives. Nonneural releasing mechanisms could involve inherent chemosensitivity of the adrenal gland itself (Comline and Silver, 1961; Steinsland *et al.*, 1970; Jones and Robinson, 1975) or humoral mechanisms secondary to the effects of asphyxia. For example, hypoxia causes a release of adrenocorticotropic hormone which mediates catecholamine release (Critchley *et al.*, 1982). Also, during forced diving, vasoconstriction of the renal bed (Jones *et al.*, 1979) associated with hypoxia and hypercapnia (Drummond and Lindheimer, 1982; Rose *et al.*, 1984) would stimulate the renin–angiotensin system causing release of catecholamines from the adrenals (Felderg and Lewis, 1965; Wilson and Butler, 1983a; Corwin *et al.*, 1985). Hence, in the presence of nonneural mechanisms, the importance of the adrenal glands compared with contributions from autonomic sympathetic vascular nerve endings to the increase in circulating catecholamines during a dive must remain a matter of speculation.

To obtain an estimate of the relative importance of the adrenal glands and autonomic vascular sympathetic nerves to the

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catecholamine increase observed during a forced dive, we compared the plasma levels of free catecholamines released in 3-min dives by adrenalectomized ducks with their sham-operated controls. Further, to estimate the importance of nonneural mechanisms stimulating the release of catecholamines from the adrenal glands, we also compared these values with those obtained in adrenal-denervated ducks and their sham-operated controls.

MATERIALS AND METHODS

Animals. The 35 ducks used in this study were 2- to 3-month-old male white Pekins (*Anas platyrhynchos*) ranging in mass from 2 to 3.5 kg. They were kept indoors at 22°C on a 12-hr light/dark cycle with food and water supplied *ad lib.* Seven ducks were adrenalectomized and six were sham-operated following the procedure used for adrenalectomy. In eight ducks, adrenal glands were denervated while in seven others, sham-operation was performed following the procedure for adrenal denervation. Seven ducks were left intact.

Major surgical procedures. The surgical techniques used for adrenalectomy and sham-operation were essentially those described by Thomas and Phillips (1975). A major difference was that the interval between the two laparotomies was 1 week instead of 1 or 2 days. Also, a deeper plane of anesthesia was used (10 mg/kg body wt iv of sodium pentobarbital (Somnotol, MTC) followed by 8 mg/kg every ½ hr). Finally, the bird was tidally ventilated with pure O_2 as surgery violated the air sacs and caused a decrease in respiratory efficiency. Before the second laparotomy, 2 mg/kg body wt of prednisone was given orally to the ducks. Recovery after the end of surgery was at least 2 weeks, which allowed the bird to regain presurgical body weight. The average body mass of adrenalectomized (ADX) and sham-adrenalectomized (SH-adx) ducks was 2.9 ± 0.2 and 2.8 ± 0.3 kg before surgery, respectively, and 2.6 ± 0.3 and 2.7 ± 0.2 kg at the end of the recovery period. Hematocrits were 37.2 ± 0.5% for SH-adx and 35.8 ± 0.8% for ADX at the end of the recovery period. Because the adrenocortex was removed, ADX ducks were given prednisone orally (2 mg/kg body wt daily) and salt water (0.8% NaCl) to drink after the second laparotomy.

The innervation of the adrenal glands has been described in the fowl by Freedman (1968). The surgical technique used for adrenal gland denervation and sham-operations was that used by Mangalam *et al.* (1987). In order to facilitate access to the nerves, the lumbar vein was ligated and cut in both adrenal denervated (DNX) and sham-denervated (SH-dnx) ducks. To make certain that all nerves had been sectioned, the adrenal glands were freed from the surrounding tissue (connective tissue, vena cava, aorta, and testes), and maintained in place only by the adrenal veins and arteries. The time for recovery after surgery was 2 to 3 weeks. The average body weight of DNX and SH-dnx were, 2.6 ± 0.1 and 2.7 ± 0.2 kg before surgery, respectively, and 2.6 ± 0.1 and 2.6 ± 0.1 kg at the end of the recovery period. At this time, the hematocrit was 37.2 ± 0.9% for DNX and 36.3 ± 1.9% for SH-dnx.

All the ducks were killed with an overdose of anesthetic at the end of the series of experiments except for one ADX which was maintained on food and salt water only and killed 2 years later. The successful removal of the adrenal glands in ADX and section of adrenal nerves in six DNX was checked *postmortem*.

Diving protocol. Cannulation of a brachial artery was done under local anesthesia (Xylocaine 2% Astra) at least 1 day before any dives. The tip of the arterial cannula was advanced until it lay near the junction of the brachial artery and aorta.

The bird was placed in the sitting position and secured with tape to an operating table. The electrocardiogram leads were inserted subcutaneously, one above the left thigh, the other below the right shoulder, while a ground lead was attached to the right foot. The arterial cannula was connected to the blood pressure transducer. This cannula was also used to take blood samples before and at 3 min submergence, which interrupted blood pressure recording. The duck was left undisturbed for 15 min before an experiment started. Ducks were dived by lowering their head gently into a beaker of cold water (16–20°C). Samples of arterial blood were taken anaerobically in 1-ml heparinized plastic syringes before and after 3 min of forced diving.

Measurement and analysis of physiological variables. Free catecholamines in the plasma were measured by HPLC, following the technique described by Mangalam *et al.* (1987). Plasma levels of dopamine do not change significantly during the dives (Mangalam *et al.*, 1987), so only the values for norepinephrine and epinephrine are reported.

After the postoperative recovery period plasma Na^+ and K^+ concentrations were analyzed in adrenalectomized ducks and their sham-operated controls, using a flame photometer (Instrumentation Laboratory, Inc., Boston, MA). Plasma Cl^- was measured by isometric titration with a Buckler digital chloridometer (4-2500, Fort Lee, NJ), and plasma glucose using an enzymatic assay kit (No. 16-UV Sigma, St. Louis, MO). Arterial pH (pH_a) was determined using an Instrumentation Laboratory 813 PH/blood gas analyzer.

ECG and arterial blood pressure signals were displayed on a chart recorder (Physiograph 6, E&M Instrument Co., Inc., Houston, TX).

Analysis of data. Comparisons were made between intact ducks and sham-operated controls to estimate the effects of surgical and postsurgical trauma. Comparison between SH-adx and ADX was made to estimate the effects of the adrenalectomy per se, and comparison between SH-dnx and DNX to establish the effects of the adrenal denervation per se. A paired *t* test was used to test the difference between pre-dive and 3-min dive blood pressures. For comparisons between more than two values, ANOVA and Newman–Keuls tests were used. A significant difference between two values was assumed if $P < 0.05$. In the text and graphs values are represented by their means ± standard error of the mean.

RESULTS

The main differences in pre-dive levels of plasma catecholamines among the five groups of ducks were the absence of plasma epinephrine (EP) in ADX (Fig. 1A) and significantly lower plasma levels of norepinephrine (NE) and epinephrine (EP) in DNX (Fig. 2A). A significant increase in plasma catecholamine levels was observed after 3 min diving in all the five groups (Figs. 1B and 2B) except for plasma EP in DNX ducks. Furthermore, no EP was detectable in the plasma of ADX ducks after 3 min submergence as was the case pre-dive. During the dive, levels of catecholamines in ADX and DNX were significantly lower than those found in their sham-operated controls. After 3 min diving, the levels of NE and EP in SH-adx were significantly higher than those in the intact ducks, but no differences were observed between SH-dnx and intacts. No significant differences in the diving levels of NE and EP were observed between ADX and DNX. No difference in diving levels of plasma NE was observed between SH-adx and SH-dnx, but diving levels of plasma EP in SH-adx were higher than in SH-dnx.

The heart rate dropped significantly during the dive in all five groups (Fig. 3). There was no significant difference in pre-dive or dive heart rate between intact, SH-adx, SH-dnx, and DNX. However, pre-dive and dive heart rate of ADX were significantly higher than those of intact, SH-adx, SH-dnx, and DNX (Fig. 3).

In all five groups the mean arterial blood pressure dropped significantly after 2 min diving. No significant differences in the pre-dive or dive values were observed among the five groups (Fig. 4).

There was no difference between ADX and SH-adx in plasma levels of Na^+ (138 ± 1.4 and 137 ± 0.9 meq/liter, respectively), K^+ (3.7 ± 0.2 and 3.1 ± 0.2 meq/liter, respectively), glucose (155 ± 18 and 197 ± 10 mg/100 ml, respectively), or in arterial pH (7.38 ± 0.02 and 7.42 ± 0.02, respectively).

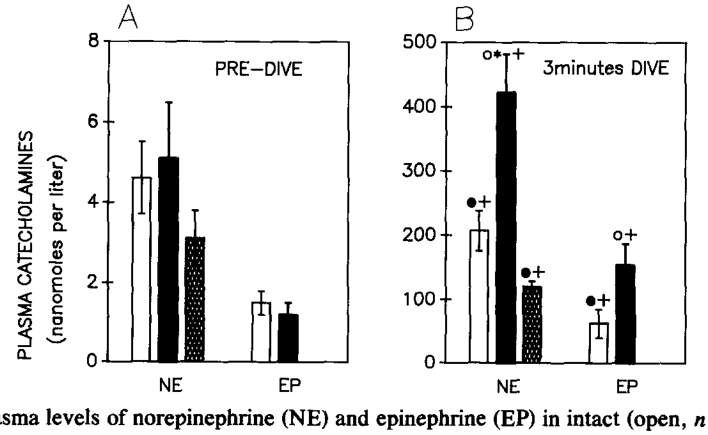

FIG. 1. Plasma levels of norepinephrine (NE) and epinephrine (EP) in intact (open, $n = 5$), sham-operated (filled, $n = 5$), and adrenalectomized (cross-hatched, $n = 6$) ducks before (A) and after 3 min forced diving (B). Significantly different from intact ducks, ○; from SH-adx, ●; from ADX, *; from pre-dive value, +.

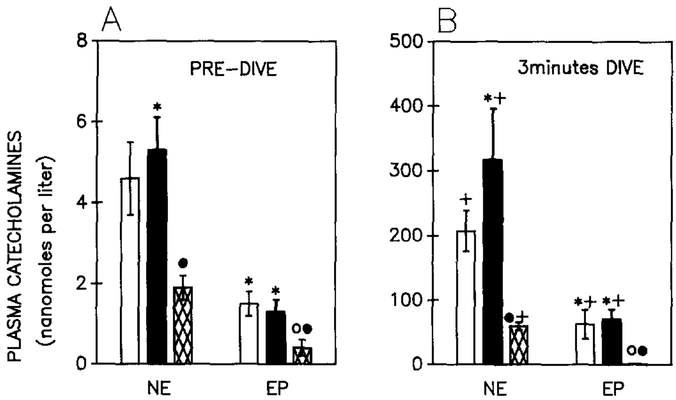

FIG. 2. Plasma levels of norepinephrine (NE) and epinephrine (EP) in intact (open, $n = 5$), sham-operated (filled, $n = 7$), and adrenal-denervated (cross-hatched, $n = 8$) ducks before (A) and after 3 min forced diving (B). Significantly different from intact ducks, ○; from SH-dnx, ●; from DNX, *; from pre-dive value, +.

However, the plasma level of Cl^- in ADX (105 ± 0.9 meq/liter) was significantly higher than in SH-adx (102 ± 0.9 meq/liter).

DISCUSSION

Plasma levels of catecholamines found in ADX showed that during the dive 100% of E and most of the NE (70%) were released by the adrenal glands. The rest must have come from the discharge of autonomic sympathetic nerves innervating the vasculature, which are strongly stimulated during the dive (Jones *et al.*, 1979). Adrenal denervation also significantly decreased plasma catecholamine levels during dives. Plasma E and NE during the dive fell by 98 and 81% of the sham-denervated values, respectively, which was greater than the 90 and 50% reduction in E and NE found by Mangalam *et al.* (1987). This discrepancy could be due to a regrowth of adrenal nerve fibers in Mangalam's adrenal denervated ducks. This would trigger a small release of adre-

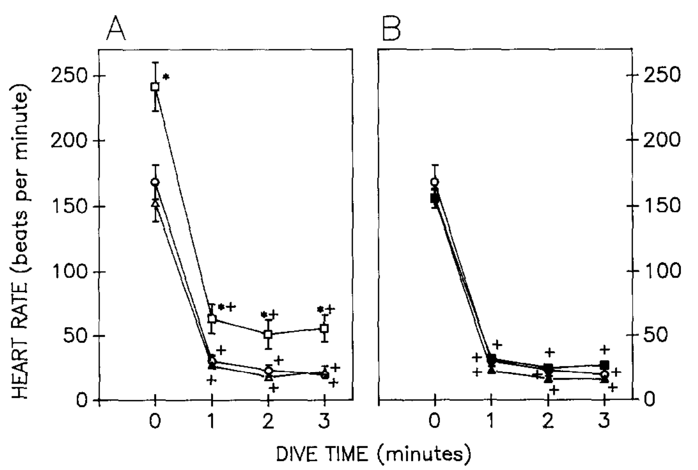

FIG. 3. Heart rate during forced dives. (A) Intact (open circle, $n = 7$), sham-adrenalectomized (open triangle, $n = 6$), adrenalectomized (open square, $n = 7$). (B) Intact (open circle, $n = 7$), sham-adrenal denervated (filled triangle, $n = 7$), adrenal denervated (filled square, $n = 8$). Significantly different from the other groups, *; from pre-dive value, +.

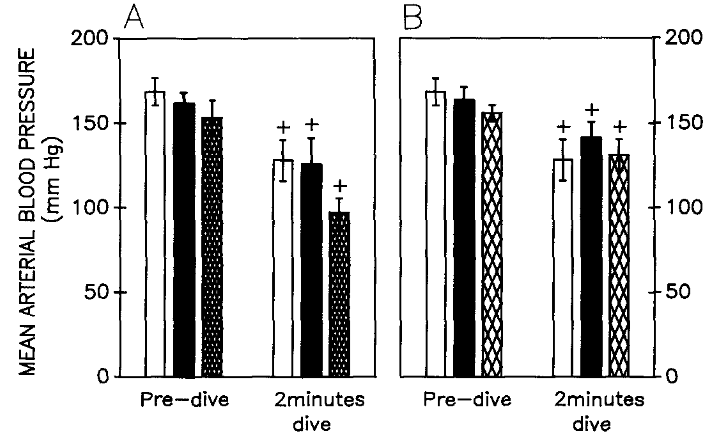

FIG. 4. Mean arterial blood pressure before and after 2 min forced diving. (A) Intact (open, $n = 5$), sham-operated (filled, $n = 6$), and adrenalectomized (cross-hatched, $n = 7$) ducks before and after 3 min forced diving. (B) Intact (open, $n = 5$), sham-operated (filled, $n = 7$), and adrenal denervated (cross-hatched, $n = 8$) ducks before and after 3 min forced diving. Significantly different from pre-dive value, +.

nal catecholamines during forced diving. In order to prevent any regrowth in the present study, 3 to 5 mm of the length of the adrenal nerves was removed. Since DNX ducks did not release more NE than ADX ducks during the dive, and there was also no increase of EP in the DNX ducks during dives, then adrenal catecholamine release during dives was neural and nonneural mechanisms did not appear to play any role.

Plasma levels of NE and E measured at rest and after 3 min submergence in intact ducks were similar to values found in resting and diving intact ducks by others (Sturkie *et al.*, 1970; Huang *et al.*, 1974; Hudson and Jones, 1982; Wilson and Butler, 1983a; Mangalam *et al.*, 1987). After adrenalectomy E was not detected in the plasma at rest, which agrees with results of Butler and Wilson (1985) and Wilson and Butler (1983b, c). The higher NE and EP levels during dives in SH-adx compared with intact ducks may have been due to surgical or postsurgical stress. However, diving NE and EP levels in SH-dnx were similar to those of intact ducks and there was also no increase in predive NE and EP in SH-adx, mitigating against any stress factor. In fact, diving levels of NE and EP in our intacts and shams were within the usual range of variability (Hudson and Jones, 1982). Even so, comparing ADX and DNX with intact ducks rather than their shams has little effect on our overall conclusion: the adrenal glands are a major source of plasma catecholamines in dives (100% of EP and 40 to 80% of NE).

During forced dives, all groups of ducks showed bradycardia and hypotension. Certainly it is remarkable that, during dives, such large differences in plasma catecholamines between DNX and SH-dnx were not reflected by any marked differences in their cardiovascular performance. Furthermore, diving heart rate of ADX was significantly higher than that of DNX though their diving levels of catecholamines were similar. Consequently, autonomic nerves play the main role in the establishment and maintenance of the cardiovascular responses to diving (Kobinger and Oda, 1969; Butler and Jones, 1971; Wilson and West, 1985). Adrenal catecholamines do not seem to participate in cardiovascular adjustments, at least not during short dives. However, Pekin ducks can be forced dived for 20 min without any physiological damage

(Jones and Furilla, 1987) and a possible role of adrenal catecholamines on maximal underwater tolerance has yet to be investigated.

The difference in cardiovascular adjustments between ADX and the four other groups of ducks during diving could be due to the lack of endogenous corticosteroid. Heart rate in resting ADX was twice that of SH-adx. The effects of corticosteroids on the cardiovascular system are not fully understood (Fowler and Chou, 1960; Lefer *et al.*, 1968; Sevy *et al.*, 1974; Rovetto, 1974; Lefer, 1974). Cardiovascular collapse, one consequence of adrenalectomy, can be a result of hypotension resulting from decreased Na^+ reabsorption in the kidneys, caused by a lack of mineralocorticoids. However, the level of Na^+ (and other plasma ions) and the pre-dive mean arterial blood pressure in ADX and SH-adx were similar and in the range of values usually recorded in ducks (Butler and Wilson, 1985; Roberts and Hughes, 1984; Shimizu and Jones, 1987) so this difference in heart rate has to involve some other mechanism. The effect of adrenalectomy on the cardiovascular system of ducks has been studied by Butler and Wilson (Butler, 1985; Butler and Wilson, 1985). They showed that in ADX there was a significant increase in heart rate associated with a decrease in blood pressure and stroke volume. They did not observe any alteration in peripheral resistance or blood and plasma volumes. Injection of β-methasone prevented hypotension in their ADX, as did prednisone in our study. However, these authors did not give any information about heart rate and stroke volume in their ADX β-methasone-injected ducks. An increase in heart rate, associated with a normal mean arterial blood pressure, may reflect an adaptation of the cardiovascular system to a decrease in stroke volume due to a decrease of myocardial performance (Lefer *et al.*, 1968; Sevy *et al.*, 1974; Rovetto, 1974)) in chronically adrenalectomized ducks supplemented with exogenous corticosteroid such as prednisone. In other words, even though prednisone maintains the ionic balance it still may not be an adequate replacement therapy for corticosterone and aldosterone, the endogenous corticosteroids.

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