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Cutaneous blood flow in the pigeon *Columba livia*: its possible relevance to cutaneous water evaporation

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Summary

The heat-acclimated rock pigeon is thought to use cutaneous water evaporation (CWE) as the 'preferred' route for heat dissipation, and this mechanism is controlled by adrenergic signaling. In the present study, we tested the hypothesis that adjustments in skin blood flow are a crucial component of this adaptation. Skin blood flow was measured by laser Doppler flowmetry and by ultrasonic flowmetry in heat-acclimated (HAc) and non-acclimated (NAc) pigeons. Skin blood flow, CWE and rectal and skin temperatures were measured under heat exposure (T_a=50 °C) or following propranolol (1.3 mg kg^{-1}) or clonidine (80 μg kg^{-1}) administration. Using laser Doppler flowmetry, we found a significant increase (1.3-fold) in skin blood flow in the dorsal skin of HAc pigeons following propanolol administration. In contrast, a significant decrease (0.7-fold) was observed in NAc birds. Injection of clonidine resulted in a significant decrease in skin blood flow in both HAc and NAc pigeons (0.4- and 0.5-fold, respectively). Heat exposure increased blood perfusion in both groups (2.5- and 1.8-fold, respectively). Using ultrasonic flowmetry, we showed that both propanolol and clonidine increase the arterial blood flow (Q_a) in HAc pigeons, while venous blood flow (Q_v) decreases. In contrast, no significant changes were found in NAc pigeons. As shown by the effect of clonidine, augmentation of skin blood flow is not a prerequisite for CWE, but normally coincides with a greater difference in arterial–venous pressure. Possible regulatory mechanisms are discussed.

Key words: Adrenergic receptor, clonidine, heat acclimation, propranolol, thermoregulation, pigeon, *Columba livia*.

Introduction

Heat-acclimated (HAc) pigeons do not resort to panting or gular fluttering for heat dissipation, even at an ambient temperature (T_a) as high as 60 °C and relative humidity (RH) of 10–15 %. In contrast, non-acclimated (NAc) pigeons start to pant at T_a=40–45 °C, RH=20–30 %, and do not tolerate prolonged (>1 h) exposure to temperatures above T_a=45 °C. It is now evident that the most prominent feature of HAc pigeons compared with NAc or cold-acclimated (CAc) birds is controlled, heat-induced cutaneous water evaporation (CWE) (Marder, 1983; Marder and Ben Asher, 1983; Marder and Gavrieli-Levin, 1987; Marder et al., 1989). In HAc pigeons, CWE is crucial for maintenance of normal body temperatures together with stable metabolic rates, even at very high ambient temperatures (Marder and Arieli, 1988), enabling the HAc pigeon to cope with, and even to breed at, such conditions (Arieli et al., 1988; Marder and Gavrieli-Levin, 1986). Despite the physiological importance of CWE in desert birds, there have been only a few attempts to investigate the mechanism involved, and it remains largely unexplored.

Inhibition of β-adrenergic receptors (β-ARs) (e.g. by propranolol) or stimulation of α_2-adrenergic receptors (α_2-ARs) (by clonidine) causes CWE to be 'switched on' in HAc pigeons, even at normothermic T_a, to levels similar to those measured in heat-exposed HAc birds (Marder and Raber, 1989; Ophir et al., 2000; Ophir et al., 1995). Moreover, these studies showed that both selective β_2-AR and non-selective β-AR agonists totally blocked pharmacologically induced as well as heat-induced CWE. The effect on heat-exposed HAc pigeons was concomitantly accompanied by intensive panting. This suggests that CWE is an adaptive trait that is subject to neural control, although humoral control cannot be excluded.

Dermal/epidermal tissue and peripheral microvasculature have both been considered as CWE effectors. (1) Dermal or epidermal tissue. Peltonen et al. (1998) showed that the skin of the HAc pigeon differs significantly from that of NAc and CAc birds. Marked differences include the presence of highly vascularized areas, a thicker epidermis (partly as a result of tissue hydration), and the unique patterns of intracellular structures such as mammalian-like keratohyalin. Furthermore, heat exposure of HAc pigeons induces structural modifications in the epidermis, reflected by the distension of extracellular spaces. (2) Peripheral microvasculature. Arieli et al. (1999) demonstrated that

microstructural adjustments in the capillary wall such as interendothelial cell gaps and fenestral openings, occurring in response to heat exposure and adrenergic manipulation at normothermic T_a, are strongly associated with CWE. The observation of Marder and Raber (1989) that both heat- and propranolol-induced CWE are accompanied by cutaneous vasodilatation suggests that this mechanism involves vasodynamic changes. Their finding that propranolol induces a vasoactive effect only in HAc pigeons suggests a possible link to CWE. Apart from this correlative association, however, there is no solid quantitative evidence for vasodynamic changes. The candidate effectors could be complementary, producing a combined synergistic effect, and adjustments in skin permeability to water might serve as a gating mechanism, with vasodynamic changes modulating the driving force of this process.

The purpose of the present study was twofold: (1) to investigate whether effective CWE requires augmented cutaneous blood flow, and (2) to determine whether the specific nature of the adrenergic control of vasomotor responses provides the means for CWE.

Our data show that although CWE is normally coupled with augmentation of skin blood flow, this relationship is merely circumstantial, and CWE is probably influenced by vasomotor adjustments designed to regulate microvascular pressure.

Materials and methods

Animals

Wild-type rock pigeons (*Columba livia* L.) weighing 235±15 g, descended from a primary stock captured in Jerusalem in the early 1980s, were used. The birds were divided into HAc and NAc groups. The HAc pigeons were maintained in an environmental chamber (883-13 model, Hotpack, Philadelphia, PA, USA) and exposed to a daily T_a cycle consisting of 4–5 h at 60 °C and 19–20 h at 25–30 °C, for each of 6 days every week, beginning at hatching. The NAc pigeons were maintained at a room temperature of 25–27 °C. Both groups were kept under a constant regime of 16 h:8 h L:D in familial cages 68×50×36 cm in size, and fed *ad libitum* on a commercial chicken feed mixture supplemented with sorghum. Drinking water was also supplied *ad libitum*.

Skin blood flow, CWE, skin temperature (T_s) and body temperature (T_b) were measured in birds from both groups at room temperature, upon acute heat exposure, and following pharmacological manipulations under normothermic conditions (room temperature). These parameters were not measured simultaneously, but in separate experiments, so as to prevent undue stress caused by lengthy handling of the birds and to reduce variability in the duration of exposure to the specific conditions, particularly to heat exposure in conscious pigeons. Blood flow in major cutaneous vessels was measured under anesthesia in tandem with CWE.

Acute experimental treatments

Acute heat exposure

(i) To measure the effect of progressive temperature elevation on CWE, T_s and T_b, the birds were placed in the environmental chamber and exposed to stepwise heating (30–60 °C T_a, in 5 °C increments; RH varied from 30–10 % with the elevation of T_a). Because NAc birds did not tolerate T_a=60 °C and were highly stressed at T_a=55 °C, the upper temperature limit for CWE measurements was set at 50 °C, and for T_b and T_s at 55 °C. Measurements were made 90 min after each temperature increment.

(ii) To determine the effect of high T_a on skin capillary blood flow, the birds were placed in an environmental chamber and exposed to two different T_a settings (25 °C, RH=50 % and 50 °C, RH=30 %). Measurements were taken inside the chamber, prior to and 90 min after the onset of heat exposure.

Pharmacological treatment

To examine the involvement of adrenergic receptors in CWE and skin blood flow, propranolol (a β-AR antagonist; Aldrich, WI, USA), or clonidine (an α_2-AR agonist; Sigma, MO, USA) was injected into the pectoral muscle with a 25-gauge hypodermic syringe at a dose of 1.3 mg kg^{-1} and 80 μg kg^{-1}, respectively. These were the minimal doses required to produce maximum CWE, determined from their respective dose–response curves (data not shown). All pharmacological manipulations were carried out under normothermic conditions. Following treatment, blood flow was measured as described below.

Measurements

Temperature

T_b and T_s were measured using a needle thermistor (Hypodermic probe no. 524, YSI, Yellow Springs, OH, USA) attached to a Tele-Thermometer (46 TUC, YSI). For T_s, the thermistor probe was lightly attached to the skin surface until a stable reading was obtained. Preliminary measurements showed that this gentle procedure affects neither local blood flow nor CWE in the measured area. The mean abdominal T_s for each bird was calculated from stable readings at two locations on the abdominal skin. For T_b, the probe was inserted 2–3 cm deep, parallel to the vent through the lower intestinal tissues. This procedure is preferred in birds because of their short rectum; use of the standard procedure for measuring rectal temperatures in mammals may cause intestinal fissure. T_b was read after approximately 3 s. The same probe was used for measuring both T_s and T_b to reduce any possible errors.

Cutaneous water evaporation

CWE was measured using a porometer (AP-4, Delta-T Devices, Cambridge, UK). This device was calibrated daily with its original calibration plate, supplied by the producer. Stable readings were automatically taken from two locations on the abdominal areas and the mean value was calculated. Resistance values were converted to CWE according to Monteith (1990), Cena and Monteith (1975) and Campbell (1977). Each value presented is the mean of the entire group of birds used in the experiment (for more detailed description, see Ophir et al., 2000).

Blood flow

Cutaneous blood flow Q was measured by laser Doppler flowmetry and ultrasonic flowmetry. Laser Doppler flowmetry measures net tissue blood perfusion in a selected area of 1 cm^2 at a depth of 1 mm. This method is based on the blood cell flow in the tissue (measuring blood cell velocity and blood cell mass to extract the flow volume) and provides flow values ($ml\,min^{-1}\,g^{-1}$) from which the relative change in tissue perfusion was calculated. Ultrasonic flowmetry measures fluid flow in individual exposed vessels and provides absolute flow values ($ml\,min^{-1}$) in the examined vessel. By integrating the data from these two distinct methods, we attempted to gain more insight into the events taking place in the cutaneous microvasculature.

Tissue perfusion was measured using an ALF21D laser Doppler flowmeter equipped with a 780 nm infrared laser diode and a 0.5 mm fiber spacing prism type probe (Advance Company, Tokyo, Japan). Measurements were made prior to and 60 min after the onset of heat exposure or after drug administration, in two areas on the dorsum of conscious pigeons, sedated by darkness. Readings were taken after at least 1 min from placing the probe on the skin, allowing the probe temperature to reach that of the skin. Only stable readings (<10 % variation over 1 min) were recorded. The blood perfusion values are the mean of the values measured every 10 s during a 2 min period. To minimize disturbances, only two measurements per animal were made: prior to and following each treatment. Consequently, no sequential dynamics of blood perfusion could be plotted.

Blood flow in single vessels was measured using the ultrasonic flowmetry method. Experiments were conducted on fully anesthetized pigeons (ketamine, 1 $mg\,kg^{-1}$, i.m.), using a T-106 blood flowmeter (TSI, Ithaca, NY, USA). The pigeon to be examined was placed on a homeothermic blanket with a control unit (Harvard Apparatus Inc., Holliston, MA, USA) set at 40 °C to maintain T_b in the normothermic range. The main arterial and venous vessels supplying the pectoral skin (a. cutanea abdominalis and v. cutanea abdominalis, respectively) were surgically exposed and detached from the skin. A U-shaped S1 probe was gently attached to each vessel and carefully fixed in the appropriate position, using a stereotactic apparatus. The entire space between the probe and the vessel was filled with Aquarius 101 ultrasound gel (Meditab, Israel). Blood flow signals were transmitted from the device to a BS-273 recorder (Gould Instrument Systems, OH, USA) and the blood flow from each vessel was measured. Readings were taken after at least 80 min from onset of anesthesia and lasted 42 min. At high ambient temperatures (>45 °C), the readings were unstable, probably because of a temperature effect on probe sensitivity or transfer of heat from the metal probe to the blood vessel, so only pharmacological manipulations under normothermic conditions are reported. Representative recordings from individual HAc and NAc pigeons are shown in Fig. 1. To calculate arterial or venous blood flow, we divided the entire period of measurement (42 min for each experiment) into 4 min units. The mean flow ($ml\,min^{-1}$) of each time unit was calculated from the area under the curve. CWE, T_s and T_b were measured separately in anesthetized pigeons. Collectively, despite its limitations (the need to anesthetize the animal), this method provided us with discrete values of blood flow in the afferent and efferent blood vessels of the measured skin area.

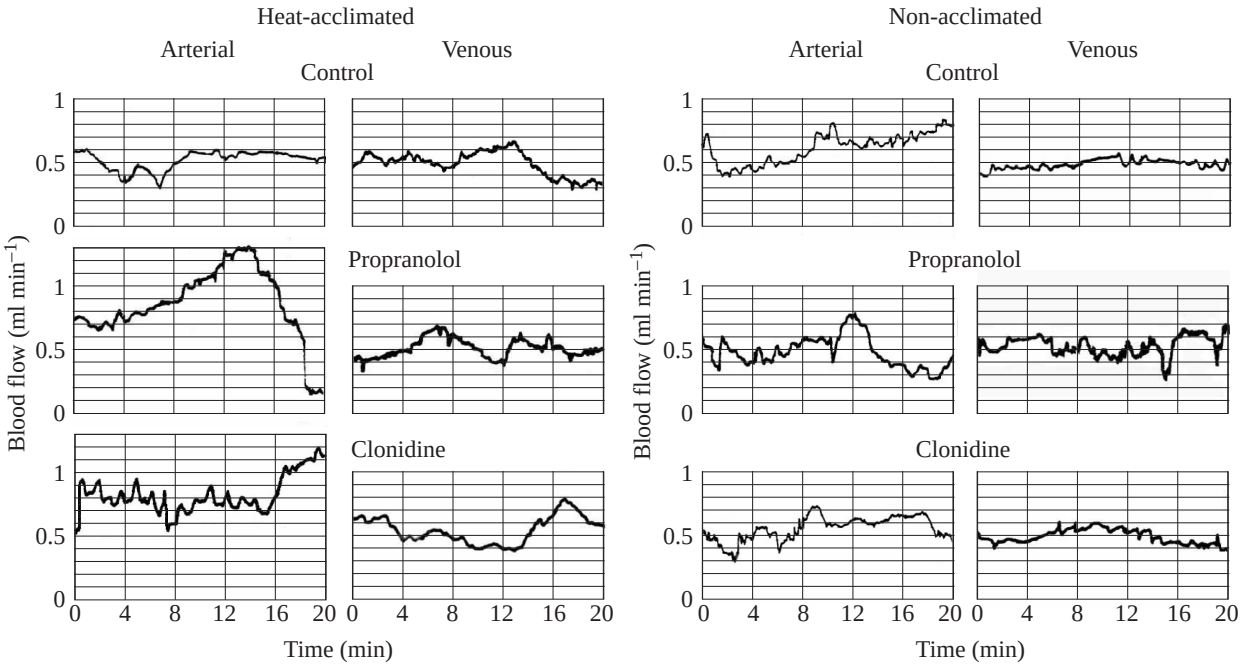

Fig. 1. Representative recordings of skin blood flow measured by the ultrasonic flowmetry method from individual heat-acclimated and non-acclimated pigeons treated with propranolol (1.3 $mg\,kg^{-1}$) or clonidine (80 $\mu g\,kg^{-1}$). The control recordings are pretreatment values.

Statistics

The significance of the differences within and between groups was assessed using two-way ANOVA for repeated measures. For *post hoc* analysis, Dunnett's control comparison analysis was used. For pairs of mean values, we used Student's two-tailed *t*-test. $P<0.05$ was considered significant; $P<0.005$ was considered highly significant. The results are presented as means ± S.E.M. All statistical analyses were performed using JMP (SAS Institute Inc., Cary, NC, USA).

Results

The effect of heat exposure on cutaneous water evaporation, T_s *and* T_b

HAc pigeons showed a gradual elevation in CWE along the entire range of T_a, reaching a maximum of 18.9 mg cm^{-2} h^{-1} at 60 °C T_a, compared with 1.2 mg cm^{-2} h^{-1} at 30 °C T_a ($P<0.005$) (Fig. 2A). The apparent elevation in CWE in the NAc pigeons was nonsignificant ($P>0.05$). The highest measurable CWE for the latter was 3.29±1.03 mg cm^{-2} h^{-1} (at T_a 50 °C), compared with 10.55±1.28 mg cm^{-2} h^{-1} for the HAc pigeons at the same T_a. Accordingly, the HAc birds exhibited relatively stable T_s and T_b values even at the highest T_a used (ΔT_s: 0.5 °C, nonsignificant; ΔT_b: 0.6 °C, $P<0.05$) (Fig. 2B). In contrast, the NAc birds showed a significant increase in T_s with elevation in T_a, peaking (44.1±0.3 °C) at 55 °C T_a (ΔT_s: 2.1 °C, $P<0.005$). A similar profile was observed for T_b, as the NAc birds underwent mild to severe hyperthermia while exposed to 55 °C T_a (ΔT_b: 1.4 °C, $P<0.005$). Both the basal T_s and basal T_b values (at 30 °C T_a) of the HAc pigeons were significantly lower compared with those of the NAc birds ($P<0.005$ for both T_s and T_b). Likewise, the T_b–T_s difference in the NAc group decreased gradually with the rise in T_a (ΔT_b–T_s=1.0±0.1 °C at 30 °C T_a *versus* 0.4±0.1 °C at 55 °C T_a, $P<0.05$), whereas in the HAc group, ΔT_b–T_s was steady with no significant differences along the entire temperature range measured (ΔT_b–T_s=0.8±0.1 °C at 30 °C T_a *versus* 1.0±0.1 °C at 55 °C T_a). Panting was not observed in the HAc pigeons during the entire experimental series presented in Fig. 2A,B, whereas NAc pigeons started to pant at 45 °C (9 birds out of 15) and continued at 50 °C T_a (15 out of 15) and 55 °C T_a (7 out of 7) (Table 1).

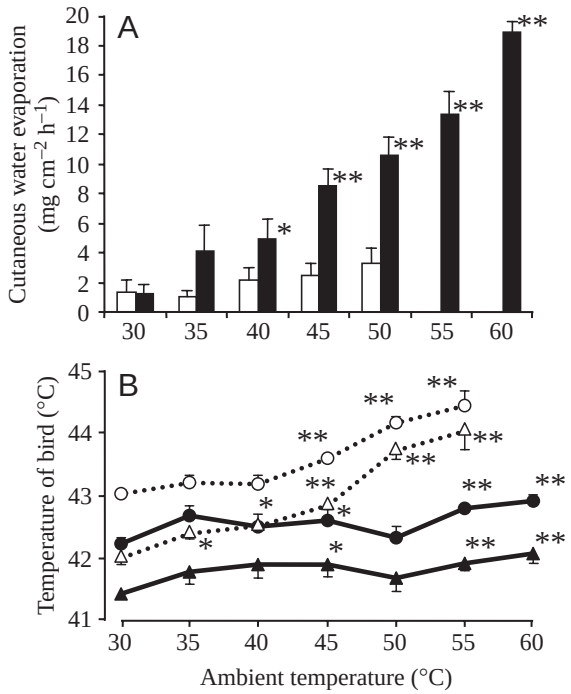

Fig. 2. The effect of heat exposure on CWE, T_s and T_b of heat-acclimated (HAc; filled bars and symbols) and non-acclimated (NAc; open bars and symbols) pigeons. The birds were exposed to different ambient temperatures (30–60 °C T_a) for 90 min prior to each measurement. (A) Cutaneous water evaporation (CWE) increased continuously with increased T_a in both NAc (N=8) and HAc (N=12) pigeons. However, this trend was much stronger in the HAc birds, reaching a CWE mean value of 10.6 mg cm^{-2} h^{-1} at 50 °C T_a and a maximum mean value of 18.9 mg cm^{-2} h^{-1} at 60 °C T_a, compared with 3.3 mg cm^{-2} h^{-1} at 50 °C T_a in NAc pigeons. (B) The different effect of T_a on T_s (triangles) and T_b (circles) in the NAc (N=7) and HAc (N=8) birds. While ΔT_s and ΔT_b at 30–55 °C T_a in the NAc birds was 2.0 and 1.4 °C, respectively ($P<0.005$), the corresponding values in the HAc birds were 0.5 and 0.6 °C, respectively (not significant). Values are the means ± S.E.M. Dunnett's analysis was used to determine the significance of difference from control (values at T_a 30 °C); *$P<0.05$, **$P<0.005$.

Drug effects on T_b *and cutaneous water evaporation*

The effects of clonidine and propranolol on T_b and CWE are presented in Fig. 3. In general, administration of clonidine or propranolol resulted in a significant drop in T_b (Fig. 3A) and an increase in CWE (Fig. 3B) ($P<0.005$). Clonidine induced a significant, hypothermic effect in the NAc birds (ΔT_b=2.8 °C, $P<0.005$). The same treatment produced a greater effect in the HAc pigeons (ΔT_b=4.5 °C, $P<0.005$). The hypothermic effect of propranolol in both the NAc and HAc pigeons was less pronounced (ΔT_b=1.1 °C and 2.6 °C, respectively, $P<0.005$). Both treatments induced a significantly greater hypothermia in the HAc group, compared with the NAc group ($P<0.005$). No significant differences were found between the drugs in their

Table 1. *The effect of ambient temperature on the evolvement of panting in heat-acclimated and non-acclimated pigeons*

	Number of birds panting (%)	
T_a (°C)	NAc	HAc
30	0 (0/15)	0 (0/20)
35	0 (0/15)	0 (0/20)
40	0 (0/15)	0 (0/20)
45	60 (9/15)	0 (0/20)
50	100 (15/15)	0 (0/20)
55	100 (7/7)	0 (0/20)

T_a, ambient temperature; NAc, non-acclimated; HAc, heat-acclimated.

Values in parentheses are number of birds.

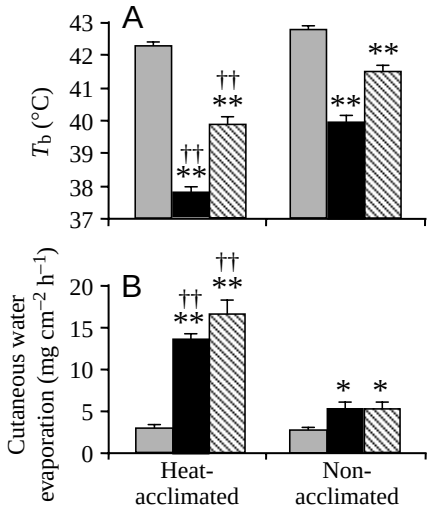

Fig. 3. The effect of propranolol and clonidine on body temperature T_b (A) and cutaneous water evaporation (CWE) (B) in heat-acclimated (HAc; N=8) and non-acclimated (NAc; N=8) pigeons. The decrease in T_b from control values in birds injected with saline (grey bars) following the administration of clonidine (80 μg kg^{-1}, i.m.; black bars) was stronger than that following propranolol (1.2 mg kg^{-1}, i.m.; hatched bars), reaching a maximum in HAc animals. The elevation in CWE values 40 min after drug administration was much greater in HAc pigeons than in NAc birds. Values are means ± S.E.M. Asterisks denote values significantly different from the control group: *P<0.05, **P<0.005; daggers denote values significantly different between the HAc and NAc groups: ††P<0.005.

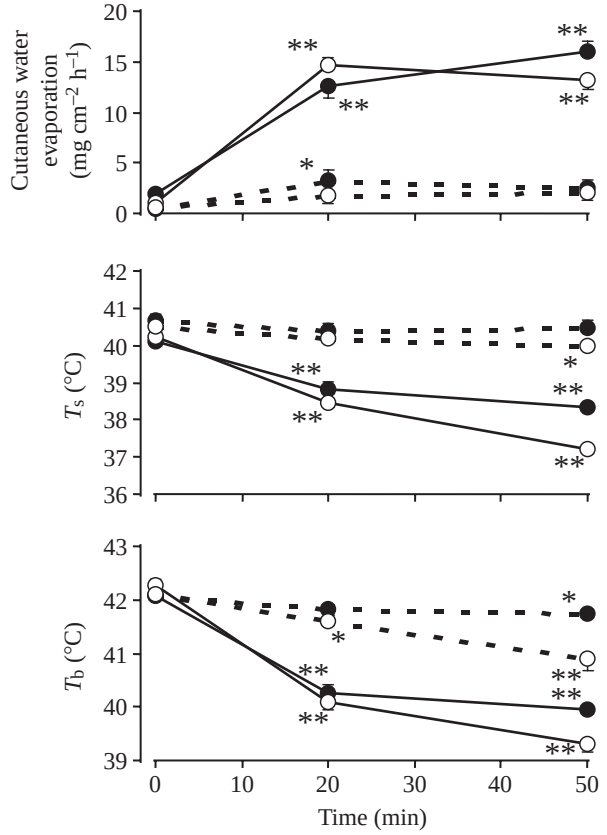

Fig. 4. Cutaneous water evaporation (CWE), skin temperature T_s and body temperature T_b in anesthetized pigeons, 0, 20 and 50 min after propranolol (filled circles) and clonidine (open circles) administration. Both drugs induced intense CWE, concomitant with a decrease in T_s and T_b in the heat-acclimated HAc (solid lines; N=7), but not in the non-acclimated NAc (broken lines; N=8) pigeons. Mild hypothermia was also observed in the clonidine-treated pigeons. Values are means ± S.E.M. Asterisks denote values significantly different from control values (at time 0) *P<0.05, **P<0.005.

effect on CWE in the HAc and NAc groups, but HAc pigeons showed significantly higher CWE following administration of either drug (P<0.005), and this effect was also observed in anesthetized birds (Fig. 4).

Measurement of subcutaneous microvascular blood perfusion

To elucidate whether CWE intensity depends on skin blood flow, both parameters were measured concomitantly, using three different stimuli: heat exposure (T_a=50 °C), propranolol and clonidine. The skin blood flow of the dorsum was measured using laser Doppler flowmetry.

As shown in Fig. 5A,D, heat exposure caused an increase in both skin blood flow and CWE in the HAc and NAc pigeons. However, the elevation of skin blood flow in the HAc group was greater (2.4-fold, P<0.005) than in the NAc group (1.8-fold, P<0.005), and the two groups were significantly different (P<0.005). Concomitantly, CWE values in the HAc pigeons increased dramatically (6.2-fold, P<0.005) compared with those of the NAc birds (2.1-fold, P<0.05), and were significantly different (P<0.005).

Propranolol administration resulted in a significant 1.3-fold increase (P<0.005) in skin blood flow in the HAc pigeons (Fig. 5B). In contrast, the skin blood flow of the NAc pigeons declined by 0.7-fold (P<0.005) following drug administration. The increase in skin blood flow in the HAc pigeons was accompanied by a marked rise in CWE (5.3-fold, P<0.005), whereas the NAc birds showed no change (P>0.05, Fig. 5E). Clonidine administration resulted in a significant decrease in skin blood flow in both the HAc and the NAc pigeons (Fig. 5C). Following clonidine administration, the skin blood flow for the HAc and NAc pigeons was 42 % and 46 %, respectively, of that obtained for the matched controls (P<0.005 and P<0.005), with no significant difference between the two groups. Fig. 5F shows a 2.7-fold elevation in CWE in the HAc birds (P<0.005), but no increase (P>0.05) was observed in the NAc group.

Arterial and venous blood flow

Arterial (Q_a) and venous (Q_v) blood flows were measured simultaneously, using ultrasonic flowmetry. Both propranolol and clonidine induced a significant increase in the skin Q_a of the HAc pigeons, while decreasing Q_v (Fig. 6A,C). The differences between Q_a and Q_v were highly significant for both treatments in the HAc pigeons (P<0.005), but not in the NAc birds (P>0.05).

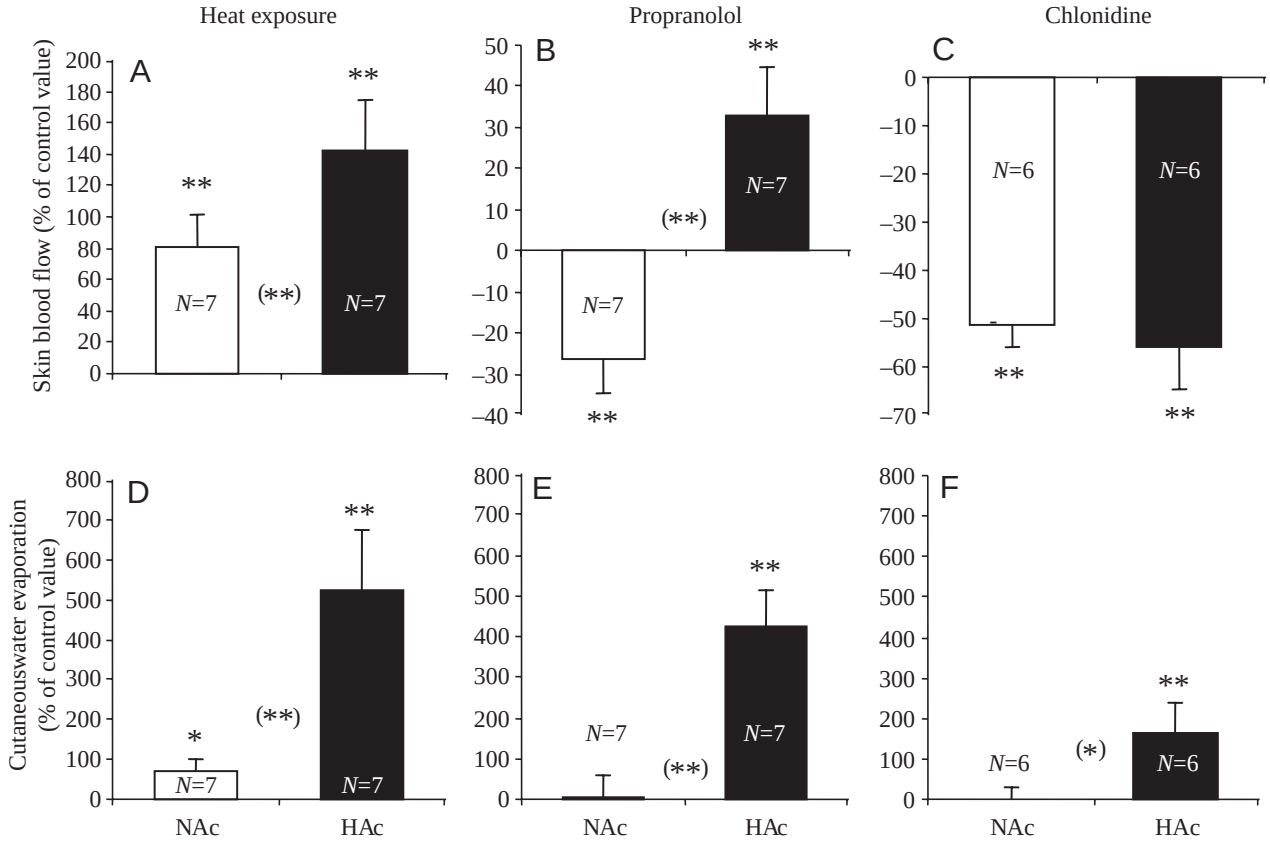

Fig. 5. Changes in skin blood flow (A–C) and cutaneous water evaporation (CWE) (D–F) in non-acclimated (NAc; open bars) and heat-acclimated (HAc; black bars) pigeons following heat exposure (T_a=50 °C) or pharmacological manipulation with propranolol or clonidine. Both skin blood flow and CWE increased in response to heat exposure in the NAc and HAc groups (A and D, respectively), and both were significantly greater in HAc pigeons. The effect of propranolol on skin blood flow (B) was dichotomous: it increased in the HAc pigeons and decreased in NAc pigeons. However, as can be seen in the HAc pigeons, clonidine dissociated skin blood flow (C) from CWE (F), reducing the former while inducing considerable CWE. Values are means ± S.E.M. *P<0.05, **P<0.005. The asterisks in parentheses indicate a significant difference between the HAc and NAc groups.

The effect of propranolol on the HAc pigeons was greater and of much longer duration than that of clonidine. Q_a gradually increased following the injection of propranolol, reaching stable values after 16–20 min. The stable blood flow was maintained to the end of the experiment (t=42 min). The effect of propranolol on Q_v was faster, the obtained values stabilizing after 4 min and remaining constant until termination of the experiment.

The effect of clonidine on Q_a in the HAc pigeons was faster than that of propranolol: steady readings were obtained 4 min after drug injection. Q_v showed a similarly quick response. The mean difference between the arterial and the venous blood flow ($\Delta Q_{a–v}$) prior to propranolol administration was not significant, whereas the mean $\Delta Q_{a–v}$ at t=20–42 min was 0.4 ml min^{-1} (P<0.005). The mean ratio of Q_a to Q_v ($Q_{a:v}$) was 0.75 prior to propranolol administration and 2.5 at t=20–42 min. No significant changes in Q_a or Q_v were found in the NAc pigeons following similar treatments (Fig. 6B,D).

Under our experimental conditions, both propranolol and clonidine individually induced a significant increase in CWE and a decrease in T_s and T_b in the HAc pigeons (Fig. 4). A weak, although significant, increase in CWE was induced by these agents in the NAc pigeons.

Discussion

Our present study shows that although CWE coincides with augmented skin blood flow under natural conditions (i.e. heat exposure), the two phenomena are not necessarily interdependent. The ability to dissociate the two effects (see Fig. 3) implies that mechanisms other than increased skin blood perfusion *per se* are involved in CWE. The integrated results of our investigation strongly suggest that hydrostatic capillary pressure in the skin serves as an important driving force in CWE, and that this component is regulated by differences in arterial:venous (a:v) resistance ratio.

During acute heat exposure in the HAc pigeons, both CWE and skin blood flow increased, whereas T_s and T_b remained relatively stable. In the NAc pigeons, the heat-induced increase in both CWE and skin blood flow was weaker, and T_s and T_b were augmented. In the NAc pigeons, ΔT_b–T_s dropped with the elevation in T_a, whereas the HAc pigeons maintained a

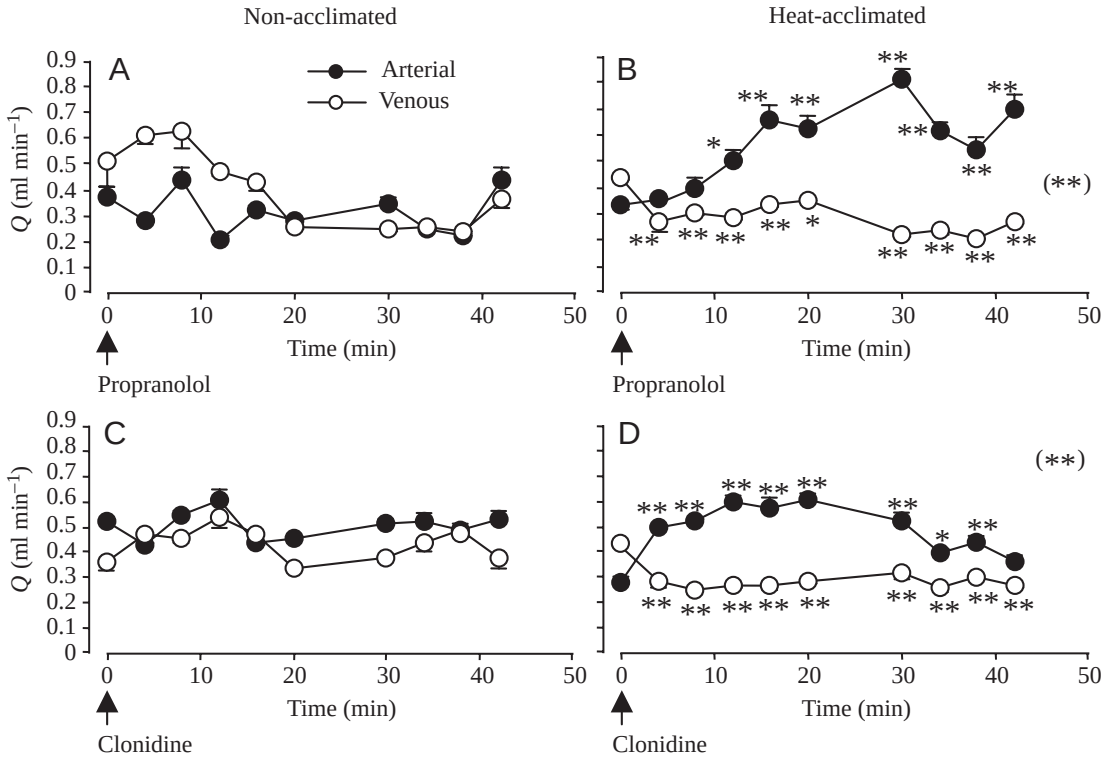

Fig. 6. The effect of propranolol (A,B) and clonidine (C,D) on arterial (Q_a) and venous (Q_v) blood flow to the abdominal skin in non-acclimated (NAc; A,C) and heat-acclimated (HAc; B,D) pigeons at room temperature. HAc pigeons responded to both treatments by an increase in Q_a and a decrease in Q_v (P<0.005). No difference in $\Delta Q_{a–v}$ blood flow was observed in the NAc pigeons (P>0.05). Values are means ± S.E.M. of 7 birds. *P<0.05, **P<0.005. The asterisks in parentheses indicate a significant difference between Q_a and Q_v values.

relatively constant $\Delta T_b–T_s$. These findings point to the strong cooling effect of CWE in the HAc pigeons.

The correlation between the dynamics of CWE and skin blood flow could imply that these two phenomena are interdependent. However, as discussed below, the finding that, following clonidine administration, blood flow was independent of CWE dynamics, rules out this possibility. Both α_2-adrenergic stimulation and β-adrenergic inhibition resulted in increased CWE, together with decreased T_b and T_s. Moreover, these effects were stronger in the HAc birds, further confirming the enhanced cooling capacity of CWE in the HAc pigeon. Another conspicuous finding in our study is the different, and to some extent antagonistic, dynamics of arterial *versus* venous blood flow ($\Delta Q_{a–v}$) to the skin (Fig. 6) that occurred in tight association with the pharmacologically induced CWE in the HAc pigeons (Fig. 4). The above findings suggest a difference in a:v resistance ratio, leading to elevated cutaneous capillary pressure and, in turn, an augmented driving force for water efflux and CWE. Interplay between α_2- and β-adrenergic signaling appears to control differences in a:v resistance.

Effect of heat exposure

The diversion of blood to the skin in homeotherms following heat exposure is well documented and acts to maintain normal T_b by enhancing heat dissipation *via* the skin (Johnson and Proppe, 1996; Jones and Johansen, 1972; Nolan et al., 1978; Wolfenson, 1983). Our finding that heat exposure increased skin blood flow supports this notion. Since water evaporation can lower surface temperature to values below the ambient temperature, CWE amplifies this ability when T_a>T_b. However, during heat challenge, HAc pigeons showed only a mild elevation in T_s, despite having a markedly higher skin blood flow. In contrast, NAc pigeons exhibited a considerable increase in T_s, with a smaller rise in skin blood flow. This difference between HAc and NAc pigeons is attributed to the former's ability to resort to CWE.

Pharmacological manipulations imply that skin blood flow is not indispensable in inducing cutaneous water evaporation

The difference between the effects of propranolol and clonidine was evident in the laser Doppler flowmetry measurements of net blood perfusion in the skin (Fig. 5). In HAc pigeons, propranolol, like heat exposure, induced an increase in skin blood flow. In contrast, clonidine, although evoking CWE, triggered a decrease in skin blood flow. The ability to dissociate CWE from skin blood flow implies that processes other than increased skin blood perfusion play a role in CWE. Not only the opposing effects of clonidine, but also the different elevation in skin blood perfusion (2.4-fold) and CWE (6.2-fold) following heat exposure or propranolol administration (1.3-fold and 5.3-fold for skin blood flow and

CWE, respectively) of HAc pigeons, supports the notion that skin blood flow is not the sole direct factor involved in the induction of CWE. Apparently, it acts in concert with other regulatory mechanisms, such as those responsible for the adaptability of skin and capillary permeability to water. Moreover, its association with CWE is conceivably derived from other, hemodynamic skin blood flow-associated changes, most likely hydrostatic capillary pressure. The effect of skin blood flow is probably instantaneous, and it may be regulated by various adrenergic-controlled factors, such as pre- and post-capillary resistance, measured in this investigation.

Control of capillary hydrostatic pressure

Greater arterial blood flow in the face of reduced venous drainage may reflect an augmented difference in a:v resistance ratio leading to greater capillary hydrostatic pressure. In turn, this may enhance water efflux from the lumen of the capillary to the epidermal tissue where it can serve as a water source for prolonged CWE. Our finding of concomitantly enhanced CWE under these conditions is in agreement with this interpretation. Our results complement previous observations of increased extravasation, manifested by the efflux of Evans Blue-labeled albumin (Arieli et al., 1999), and hydrated epidermal tissue (Peltonen et al., 1998) following exposure of HAc pigeons to acute heat. It is not clear how this augmented difference in a:v resistance is achieved. However, it is evident from our data that the hemodynamic influence of both α- and β-AR agents used in the HAc pigeons does not correspond to the effect that would be expected from our knowledge of mammalian pharmacology (Crandall et al., 1997; Koss, 1990; van Brommelen et al., 1986). On the contrary, both drugs induced augmented arterial blood flow. Interestingly, this is not the only exception to the mammalian pattern. Propranolol induces cardioacceleration in summer-acclimatized pigeons, in contrast to its suppressive effect in mammals, and in winter-acclimatized pigeons (Ophir et al., 2002). However, based on the results obtained in this investigation and our previous studies (Ophir et al., 1995; Ophir et al., 2000), we hypothesize that differences in α/β-adrenergic receptor density and affinity are involved, as illustrated in Fig. 7. The role of other vasoactive signaling pathways cannot be excluded, although this was beyond the scope of the present investigation. Light and electron microscopic findings from our laboratory (Y. Arieli, unpublished results) revealed the existence of flattened venules (comprising approx. 5 % of the total microvessels in the skin), detected exclusively in the skin of HAc heat-exposed pigeons. This observation may imply that a glomerulus-like mechanism, such as post-capillary constriction, occurring during the CWE period, contributes to the greater difference in a:v resistance ratio.

Intra/extravascular fluid passage in the skin

When calculating the amount of water evaporating from the abdominal skin (up to $0.5\,\mu l\,cm^{-2}\,min^{-1}$), we found it much lower than the mean value of ΔQ_{a-v}, normalized to the supplied skin area (approximately $10\,\mu l\,cm^{-2}\,min^{-1}$). A mechanism

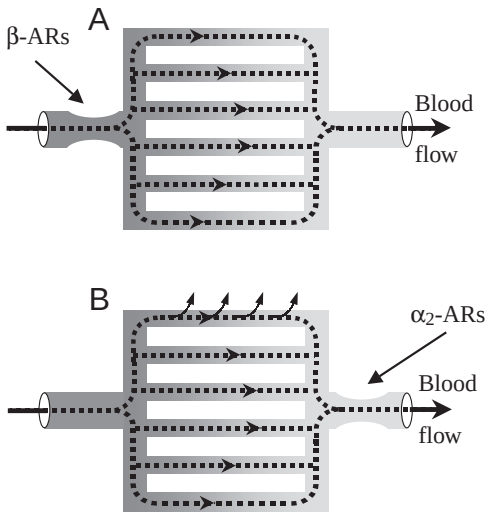

Fig. 7. Diagram of a possible mechanism for vasomotor control in the pigeon skin vasculature. We suggest that activation of β-adrenergic receptors (β-AR) constricts arterial vessels (A), while activation of α_2-ARs constricts vessels somewhere along the venous side (B). These effects can be direct or indirect. Thus propranolol (β-AR) and clonidine (α_2-AR) act jointly to raise capillary hydrostatic pressure by decreasing arterial resistance and inducing greater resistance at the venous side. The rise in pressure may induce water outflow from the capillary, thereby increasing cutaneous water evaporation.

based on intensive extravasation, counterbalanced by drainage *via* the lymphatics, could account for this apparent discrepancy.

Indeed, a considerable extravasation takes place in the skin microvasculature of the HAc pigeon. Arieli et al. (1999) found that intensive cutaneous extravasation of Evans Blue-labeled albumin occurs following heat exposure or propranolol administration in the HAc pigeon, thus providing a driving force for water efflux. Therefore, the resultant outflow should be partly negated by intensive lymphatic drainage, rather than by post-capillary reabsorption (Fig. 8). As shown previously (Arad et al., 1989), the protein content of avian plasma, especially the albumin concentration, is substantially lower than that measured in mammals. This results in low basal colloid-osmotic pressure in the capillary, thus reducing its ability to retain water. This situation would require an alternative pathway to restore large volumes of water outflow in certain tissues, namely the lymphatic system. Although we have no direct evidence for a highly developed lymphatic vessel network in pigeon skin, there is evidence for such functional, rich lymphatic networks in other tissues in the pigeon and in other birds (Berens von Rautenfeld and Budras, 1981; DeStefano and Mugnaini, 1997). However, it was argued that the fluid balance in the skin relies on lymph flow (Levick, 1995). This scenario predicts the development of a difference in a:v hematocrit. The exceptionally low whole body:venous hematocrit ratio (0.71) documented in the pigeon (Kalomenopoulou and Koliakos, 1989) supports this hypothesis. A possible

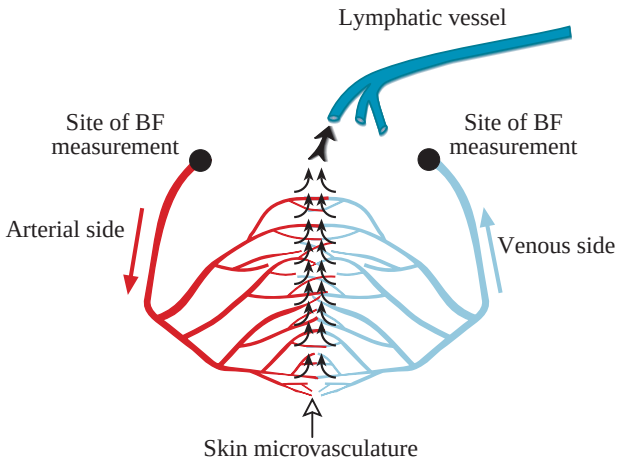

Fig. 8. Diagram of the hypothetical route of blood flow (BF; coloured arrows) in the cutaneous microvasculature accounting for the prominent ΔQ_{a-v} following chemically induced cutaneous water evaporation. As intensive extravasation occurs in the capillary bed, extensive lymphatic vasculature is responsible for the reabsorption of any excess fluid volume (black arrows).

consequence of the findings of Arieli et al. (1999) would be a drop in the colloid-osmotic pressure in the venous side of the capillary, concomitant with a rise in the perivascular interstitial colloid-osmotic pressure. In conclusion, low basal values of capillary colloid-osmotic pressure in birds might reduce the inward driving force, particularly during CWE. Alternatively, enhanced lymphatic return could remove excess water from the tissue. Augmented protein extravasation during CWE would further reinforce this route of fluid return to the circulatory system.

Conclusions

Taken together, our present findings provide a conceptual basis for the existence of a powerful driving force in the evaporative cooling mechanism of the HAc pigeon. We envisage the CWE process as orchestrated complementary changes, both in the cutaneous microvasculature and in the dermal/epidermal tissue architecture, possibly controlled by adrenergic signaling. Greater skin capillary hydrostatic pressure and increased plasma protein extravasation provide the driving force for water efflux to the epidermis. Concomitantly, in the skin, swelling of the cells in the hydrated epidermis, together with intra- and extracellular ultrastructural adjustments, result in greater water permeability, thus allowing water movement towards the skin surface (Arieli et al., 2002; Peltonen et al., 1998). In other words, structural and ultrastructural changes in the skin and endothelial openings serve as the gating component of the process, while hemodynamic events provide the driving force. These events are conceivably accompanied by a reduction in water reabsorption by postcapillaries and venules due to colloid leakage into the interstitial space. In turn, the lymphatic vascular network may be responsible for drainage of the excess water into the circulation.

Perspectives

The results of the present study, together with accumulating knowledge on ultrastructural and physiological changes in the skin and its microvasculature in heat-acclimated pigeons, allow a better and more integrated insight into one of the most highly efficient cooling mechanisms known. Yet, this investigation raises fundamental questions, such as the uniqueness or generality of this mechanism and its various components. Comparative studies on CWE in other avian species may illuminate the pattern of its phylogenetic distribution, evolutionary origin and significance.

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