CMLS, Cell. Mol. Life Sci. 62 (2005) 1881–1889
1420-682X/05/161881-09
DOI 10.1007/s00018-005-5149-5

CMLS Cellular and Molecular Life Sciences

Research Article

Circulating serotonin in vertebrates

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Received 2 April 2005; received after revision 15 May 2005; accepted 15 June 2005
Online First 22 July 2005

Abstract. The role of circulating serotonin is unclear and whether or not serotonin is present in the blood of non-mammalian species is not known. This study provides the first evidence for the presence of serotonin in thrombocytes of birds and three reptilian species, the endothermic leatherback sea turtle, the green sea turtle and the partially endothermic American alligator. Thrombocytes from a fresh water turtle, American bullfrog, Yellowfin tuna, and Chinook salmon did not contain serotonin. Serotonin is a vasoactive substance that regulates skin blood flow, a major mechanism for endothermic body temperature regulation, which could explain why circulating serotonin is present in warm-blooded species. The temperature sensitivity of human blood platelets with concomitant changes in serotonin content further supports a link between circulating serotonin and thermoregulation. Phylogenetic comparison of the presence of circulating serotonin indicated an evolutionary divergence within reptilian species that might coincide with the emergence of endothermy.

Key words, Serotonin; platelet; thrombocyte; endothermy; mammal; reptile.

Numerous studies have demonstrated the presence of serotonergic neurons in species from *Drosophila* [1] to human [2, 3]. In vertebrates, serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that plays a role for all important bodily functions, such as sleep [4, 5], food intake, mood [6], and mammalian body temperature regulation [7–9]. The anatomical organization of brain serotonergic systems is remarkably conserved among vertebrates [10]. Serotonin plays important roles in the peripheral nervous system as well. Serotonin has been shown to regulate hypothalamic-pituitary-interrenal activity in teleost fish [11], which is the teleost homolog of the mammalian hypothalamic-pituitary-adrenal axis. The hypothalamic-pituitary-adrenal axis is, at least in part, regulated by serotonin [12]. A phylogenetic study of serotonin-immunoreactive structures in the pancreas of various vertebrates has shown that, except for the American bullfrog, the investigated vertebrates (eel, turtle, mouse, rat, and guinea pig) showed serotonin-immunoreactive nerve fibers [13].

Tryptophan is the amino acid precursor of serotonin. Tryptophan hydroxylase and amino acid decarboxylase convert this essential amino acid into serotonin [14, 15]. In birds and mammals, including humans, serotonin is produced not only in the neuronal system but also independently in peripheral tissues [16, 17]. The enterochromaffin cells of the mammalian intestine synthesize serotonin and utilize it in their role as sensory transducers to activate primary afferent nerves that control bowel function and responses such as nausea, intestinal secretion, and the peristaltic reflex [18, 19]. In addition, mammalian enterochromaffin cells release serotonin into the blood, where platelets have evolved a highly efficient uptake and transport system [20–23]. Bird thrombocytes are also known to contain dense granules [24]. Thrombocytes are large nucleated blood cells and are the evolutionary precursors of mammalian platelets, which are small cells containing a number of different granules but lacking a nucleus. Thrombocytes have been shown to aggregate and participate in the hemostatic process similar to

mammalian platelets [25]. Whether thrombocytes from species other than birds contain serotonin-transporting dense granules is not known. Daimon et al. [26] did not find serotonin in tortoise thrombocytes while Pellizzon et al. [25] possibly challenged this view.

Circulating serotonin is a potent biogenic amine that exhibits strong vasoactive properties [27, 28], possibly through stimulation of serotonin receptors on endothelial cells and nitric oxide production [29, 30]. Serotonin uptake into platelet dense granules protects the mammalian organism from serotonin-induced uncontrolled, harmful vasoconstriction or vasodilation [31]. Recently, serotonin synthesis was blocked by the disruption of the non-neuronal tryptophan hydroxylase 1 gene (*tph1–/–*) in mice. The mutant mice displayed a significant drop in peripheral serotonin causing progressively abnormal cardiac activity and bleeding [32].

In homeotherms, such as mammals and birds, the blood facilitates initial rewarming of peripheral regions and a subsequent decrease in cutaneous perfusion to reduce heat loss when the environmental temperature is lower than the core temperature [33]. Serotonin plays a major role in this process. Through interaction with the endothelial cells of the blood vessels, serotonin enhances microvascular permeability and increased tissue perfusion in response to heat [34] and causes cold-induced cutaneous vasoconstriction [35]. Because the capillary system of the human microcirculation has an effective exchange area of about 1,000 m^2, vasodilation and vasoconstriction are the most effective thermoregulatory mechanisms [36, 37]. Heat dissipation during hyperthermia leads to reddening of the skin in hot and, at least initially, in cold environments. Serotonin has also been suggested to play a role in avian non-shivering thermogenesis [38].

The vasoactive substance serotonin seems to have several different functions in warm-blooded animals related to platelet aggregation, hemostasis and cardiac function. However, the absence of circulating serotonin in cold-blooded animals might support the view that serotonin is also involved in thermoregulation by regulating skin blood flow in warm-blooded animals. Thus, the goal of this study was to obtain detailed information on the phylogenetic distribution of peripheral serotonin and, based on these results, to infer the evolutionary appearance of serotonin in the circulation.

Materials and methods

Preparation of blood samples

The study of serotonin in human blood samples was approved by the Ethical Review Boards of the University of British Columbia and Vancouver General Hospital and was conducted in accordance with the Declaration of Helsinki. Human blood was taken from volunteers who provided written informed consent for participation. All procedures involving animals conformed to University of British Columbia animal care guidelines. Fresh whole blood samples from Steller sea lions (*Eumetopias jubatus*), an African bush elephant (*Loxodonta africana*), an Asian elephant (*Elephas maximus*), a Peking duck (*Anas platyrhynchos*), glaucous-winged seagulls (*Larus glaucescens*), leatherback sea turtles (*Dermochelys coriacea*), a green sea turtle (*D. coriacea*), a red-ear slider (*Pseudemys scripta*), American alligators (*Alligator mississippiensis*), American bullfrogs (*Rana catesbeiana*), and Chinook salmon (*Oncorhynchus tshawytscha*) were kindly provided by investigators working with these animals. The sea lions were housed at the Vancouver Aquarium. Elephant blood was sent from the Calgary Zoo. Ducks, seagulls, turtles, alligators, and bullfrogs were housed in animal care facilities at the University of British Columbia. Salmon were produced and maintained in fresh water in a secure, physically contained facility at Fisheries and Oceans Canada, West Vancouver Laboratory. Blood from Yellowfin tuna (*Thunnus albacares*) and a swordfish (*Xiphias gladius*) were obtained on a cruise facilitated by the National Oceanic and Atmospheric Administration (chief scientist R. Brill) north of the island of Hawaii.

Human blood was drawn into Vacutainer tubes containing sodium citrate (3.8% final concentration; Beckton Dickinson, Franklin Lakes, N. J.). Animal blood was drawn into syringes and immediately transferred to Vacutainer tubes with 3.8% sodium citrate or acid citrate dextrose anticoagulant.

Human, sea lion, elephant, frog, tuna, and salmon blood samples were centrifuged at 200 g for 12 min at room temperature. Avian and reptilian thrombocytes were obtained from whole blood after sedimentation of the red blood cells for 10 min on the bench (1 g). Platelet-rich plasma (PRP) or thrombocyte-rich plasma (TRP) was removed with a transfer pipette and fixed for the immunocytochemical microscopy assay or separated into cell pellets and plasma for high-pressure liquid chromatography (HPLC) as described below. Fixation with paraformaldehyde [PFA; 2% final concentration in phosphate-buffered saline (PBS; 50 mmol/l $NaH_2PO_4 \cdot H_2O$, 5 mmol/l KCl, 1.5 mmol/l $MgCl_2 \cdot 6H_2O$, 80.1 mmol/l NaCl; pH 7.4)] was carried out at room temperature for 30 min, after which samples were frozen at –80 °C until use.

Visualization of serotonin by immunofluorescence microscopy

Phase contrast or differential interference contrast (DIC) and fluorescence microscopy were performed with a Zeiss Axioplan 2 microscope with appropriate attachments using a ×100/1.3 NA Plan Neofluar oil immersion objective. The microscope was equipped with a digital video camera (Digital Video Camera Company, Austin, Tex.). Fixed platelets or thrombocytes were permeabilized

with Triton X-100 (0.2% final concentration) and washed with PBS before addition of the serotonin-specific monoclonal mouse anti-human antibody (Dako Diagnostics, Mississauga, Canada). After incubation and another washing step, the secondary Alexa488-labeled goat anti-mouse IgG_1 (Cedarlane, Mississauga, Canada) was added at a dilution of 1:40. Fluorescence and phase contrast or DIC microscopy were performed at the same time. Negative control samples for fluorescence microscopy were prepared by omitting the incubation with the serotonin-specific antibody. According to the manufacturer's specification, the serotonin-specific antibody recognizes serotonin in formalin-fixed human samples; since this antigen can be expected to be the same even in different species, reactivity of the anti-human antibody with serotonin in fixed samples of all species was expected [39, 40].

HPLC with electrochemical detection

Serotonin concentrations in the platelet or thrombocyte pellets were measured with HPLC using an XTerra reverse phase column (Waters Corporation, Milford, Mass.) in conjunction with an electrochemical detector (Alliance 2690-464, Waters). For HPLC, PRP and TRP were prepared as described above, by centrifugation or sedimentation and subsequent aspiration of the PRP or TRP. After centrifugation of the PRP and TRP at 1000 g for 15 min at 4 °C, the plasma supernatant was completely removed from platelets or thrombocytes. The residual pellet was immediately frozen at –80 °C until use. Thawed pellets were resuspended in PBS containing 0.5 mg/ml cysteine hydrochloride (Fisher Scientific, Whitby, Canada) to protect serotonin from oxidation. Trichloroacetic acid (TCA; 0.2 N final concentration) was added to all samples to precipitate the proteins. The samples were centrifuged and the supernatants were filtered and injected into the HPLC system. Isocratic flow at 0.25 ml/min was used with a mobile phase consisting of 5% methanol, 3% acetonitrile, and 92% phosphate buffer (100 mmol/l NaH_2PO_4, 0.5 mmol/l EDTA, pH 3.8 adjusted with tetrafluoroacetic acid). Standards for serotonin were prepared by dissolving different amounts of serotonin hydrochloride (Sigma, Oakville, Canada) in PBS. The standards were treated with cysteine and TCA analogous to the samples. Under these conditions, the retention time for serotonin was 5.2 ± 0.3 min at 4 °C, and no interference with other substances was observed. The detector voltage was set to 650 mV, and the power range was 10 mA with the direct current (DC) setting.

Electron microscopy of whole mounts

A drop of fixed PRP or TRP was placed on a Formvar-coated grid (Electron Microscopy Sciences, Fort Washington, Pa.). After evaporation of the water, the grid was placed in a transmission electron microscope (Philips EM400, Eindhoven, The Netherlands) and viewed with ×5200 or ×1400 magnification for human platelets or frog thrombocytes, respectively.

Results

Immunocytochemical demonstration of serotonin in platelets and thrombocytes

Microscopic inspection of platelets and thrombocytes fluorescently labeled with a specific serotonin antibody revealed that serotonin is present in intracellular compartments in human platelets as well as in seagull, alligator, and sea turtle thrombocytes (fig. 1, fig. 2A–D). Phase contrast or DIC micrographs (first column in figs 1, 2) show the morphology of human platelets as well as seagull, alligator, and turtle thrombocytes. The binding of

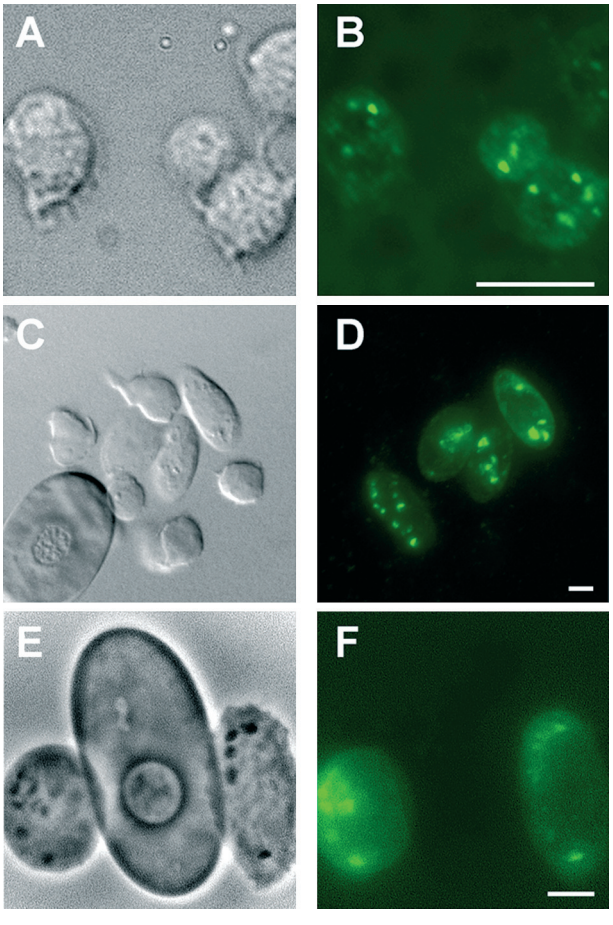

Figure 1. Immunocytochemical evidence for serotonin in human platelets and in seagull and alligator thrombocytes. Platelets and thrombocytes were isolated from whole blood and incubated with a specific serotonin antibody and a fluorescently labeled secondary antibody. DIC (*A*, *C*) or phase contrast (*E*) micrographs show the morphology of human platelets (*A*) as well as seagull (*C*) and alligator (*E*) thrombocytes, erythrocytes and leukocytes. Platelets (*B*) and thrombocytes (*D*, *F*) showed serotonin-specific fluorescent labeling. The bright dots indicate the presence of serotonin in dense granules. Scale bars, 10 µm.

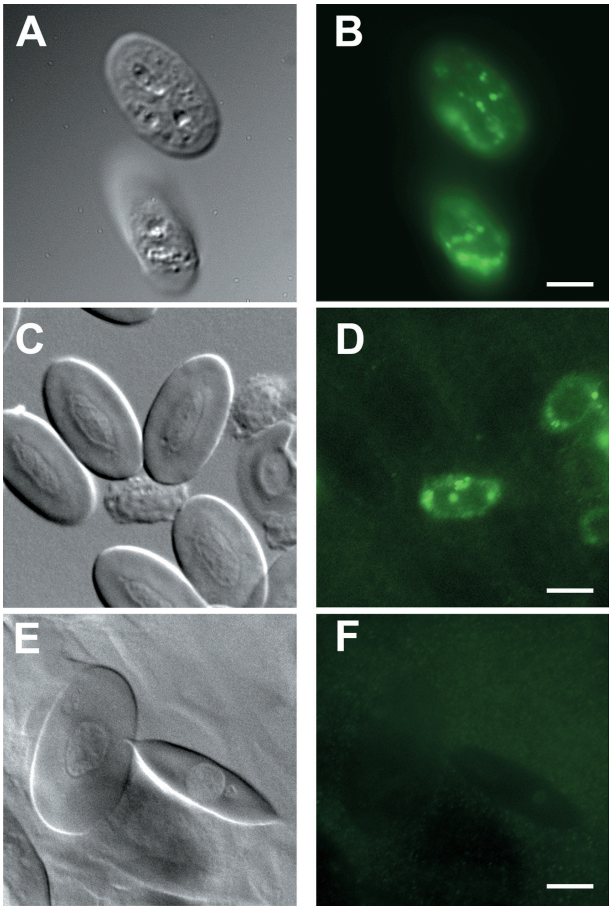

Figure 2. Immunocytochemical visualization of serotonin in thrombocytes of sea turtles but not a fresh water turtle. The DIC micrographs (*A*, *C*, *E*) show large nucleated thrombocytes and erythrocytes. Serotonin-specific fluorescence was detected in leatherback sea turtle (*B*), green sea turtle (*D*), but not red-ear slider (*F*) thrombocytes. Erythrocytes do not contain serotonin. Scale bars, 10 µm.

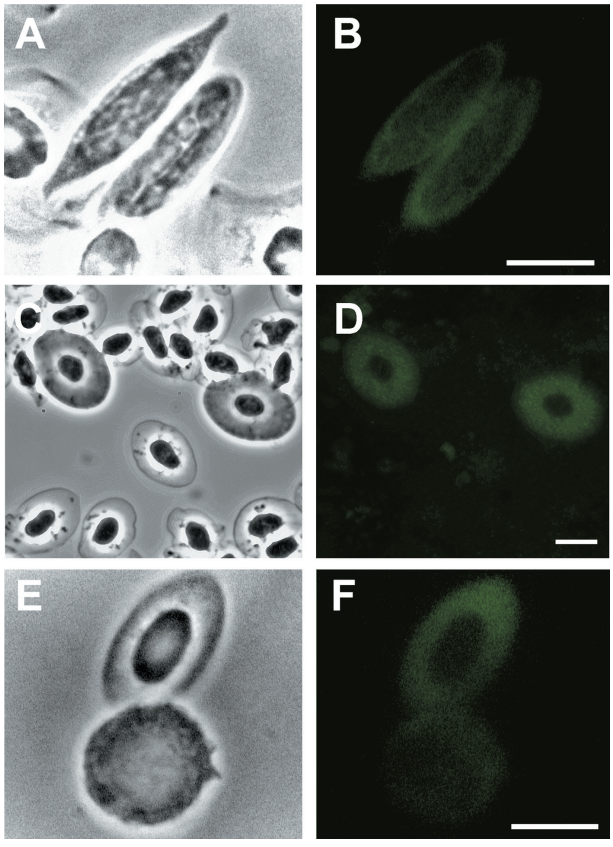

Figure 3. Immunocytochemical evidence that bullfrog, tuna, and salmon thrombocytes do not transport serotonin. The phase contrast micrographs (*A*, *C*, *E*) show large nucleated thrombocytes and erythrocytes. No serotonin-specific fluorescence was detected in frog (*B*), tuna (*D*), or salmon (*F*) thrombocytes. Scale bars, 10 µm.

the fluorescently labeled antibody to serotonin inside granules is visualized in the fluorescence micrographs as bright dots. We have previously used this assay for serotonin determination in human platelets [41, 42]. In this study, serotonin-specific fluorescent labeling was shown not only for human platelets (fig. 1B) but also for seagull (fig. 1D), alligator (fig. 1F), and sea turtle (fig. 2B, D) thrombocytes. Large, nucleated red blood cells in the alligator and turtle samples (fig. 1E, red blood cell between two thrombocytes) did not show any serotonin-specific fluorescence. This indicates that thrombocytes are specialized for serotonin transport, as are their evolutionary descendants, the mammalian platelets. Interestingly, the fresh water turtle did not show serotonin-specific labeling (fig. 2F), suggesting a split between sea and fresh water turtles. The images shown are representative for platelets from 30 humans and thrombocytes from 4 seagulls, 6 alligators, 3 sea turtles, and 1 fresh water turtle.

Bullfrog, tuna, and salmon thrombocytes also showed no serotonin-associated fluorescence (fig. 3). The phase contrast micrographs (fig. 3A, C, E) show large nucleated thrombocytes, erythrocytes, and a leukocyte (fig. 3E). Only faint background fluorescence is visible in the frog (*B*), tuna (*D*), and salmon (*F*) thrombocytes, due to non-specific secondary-antibody binding unrelated to serotonin; the red blood cells are completely unlabeled and contain aggregated hemoglobin. The images are representative for thrombocyte samples obtained from two frogs, eight tuna, and two salmon. The absence of serotonin-specific immunofluorescence might be attributed to the failure of the anti-human antibody to recognize the antigen in fixed frog, tuna, and salmon thrombocytes. HPLC analysis and whole-mount electron microscopy were therefore performed to support the results obtained with the immunocytochemical assay.

Table 1. Blood serotonin in vertebrates

Class	Species		Number of individuals	Serotonin concentration (ng/mL PRP or TRP)
	Name	Scientific name		
Mammals	human	*Homo sapiens*	30	880 ± 200
	sea lion	*Eumetopias jubatus*	1	1050
	elephant	*Loxodonta africana*	1	660
		Elephas maximus	1	1260
Birds	Peking duck	*Anas platyrhynchos*	1	412
	glaucous-winged seagull	*Larus glaucescens*	4	420 ± 100
Reptiles	American alligator	*Alligator mississippiensis*	6	580 ± 80
	leatherback sea turtle	*Dermochelys coriacea*	2	890 ± 70
	green sea turtle	*Chelonia mydas*	1	670
	red-ear slider	*Pseudemys scripta*	1	0
Amphibians	American bullfrog	*Rana catesbeiana*	2	0
Fish	Yellowfin tuna	*Thunnus albacares*	8	0
	swordfish	*Xiphias gladius*	1	0
	Chinook salmon	*Oncorhynchus tshawytscha*	2	0

Quantitation of serotonin in platelets and thrombocytes with HPLC.

HPLC is a well-established method for quantitative serotonin measurements [43]. Here it was used together with electrochemical detection (ECD) to quantify serotonin in platelets and thrombocytes. Representative examples of serotonin-positive chromatograms of human, alligator, and sea turtle are shown (fig. 4). The injection volumes were adjusted between 5–20 µl to assure that results were not off-scale. Fresh water turtle, frog, tuna, and salmon samples did not contain a component eluting at the retention time of serotonin of 5.2 ± 0.3 min. Platelets from marine mammals also contain serotonin, which was determined by immunofluorescence (data not shown) and HPLC-ECD. The serotonin content of sea lion platelets was 1050 ng/ml. The average concentrations of circulating serotonin in different species are summarized in table 1. All samples were analyzed in duplicate.

Visualization of dense-granule-calcium on whole mounts.

Platelets contain serotonin in dense granules together with calcium, ATP, ADP, and phosphate [23]. The electron density of calcium characterizes dense granules in transmission electron micrographs of thin sections [24] or whole mounts of cell suspensions [44]. The visualization of dense granules on whole mounts with electron microscopy was used in this study as a third independent indicator of the presence of dense granules (fig. 5). Whole mounts of human platelets and frog thrombocytes were compared. Human platelets contain numerous dense granules (fig. 5A), which are absent in frog thrombocytes (fig. 5B). These data provide additional evidence that frog thrombocytes do not contain dense granules and are therefore unlikely to contain serotonin.

Phylogenetic history of circulating serotonin

Figure 6 depicts the phylogenetic comparison of species based on fossil records [45]. The separation of living reptiles and mammals 310 million years ago is a calibration point where fossil time and molecular clocks result in very similar estimates. Serotonin appears in the circulation in partially endothermic (American alligator) or truly endothermic reptiles (leatherback sea turtle) that evolved approximately 310 million years ago. Blood from all evolutionary younger species that we analyzed also contains serotonin.

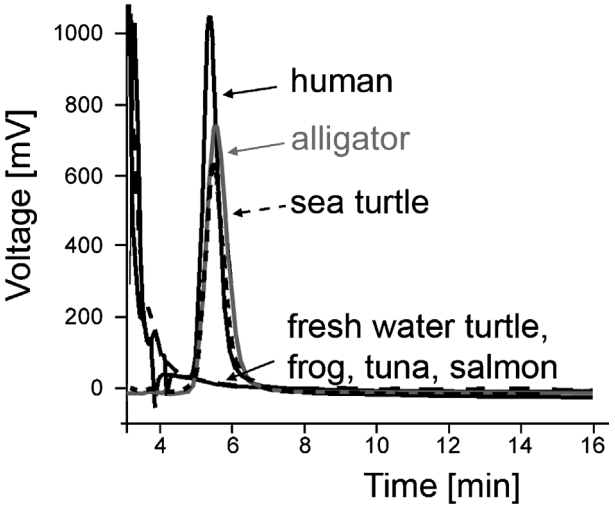

Figure 4. Quantitative determination of serotonin with HPLC. Serotonin levels in isolated platelets or thrombocytes were determined chromatographically to confirm the results obtained with immunofluorescence. Human, alligator, and sea turtle blood contain serotonin, but that of fresh water turtle, frog, tuna, or salmon do not. The results are summarized in table 1.

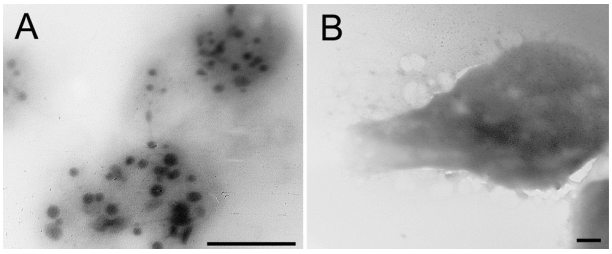

Figure 5. Visualization of calcium-containing dense granules on whole mounts. Human platelets (*A*) contain numerous electron-dense granules, indicated by the dark spots. Dense granules store calcium together with serotonin. In contrast, frog thrombocytes (*B*) are devoid of these intracellular structures. Scale bars, 2 µm.

Temperature-dependent changes of serotonin in human platelets.

If human platelet serotonin plays a role in temperature control, exposure of human platelets to different temperatures should affect the serotonin content. At the physiologic temperature of 37 °C, platelets maintain a discoid morphology (fig. 7A) [44]. Cooling causes cytoskeletal rearrangements that lead to an activated 'spiny sphere' morphology (fig. 7C). The severe effect of cooling on the morphology of human platelets is paralleled by an increase in serotonin-specific fluorescence at low temperature (fig. 7D). We have previously shown that platelets are sensitive to hypothermia and take up serotonin when exposed to low temperature [46].

Discussion

The availability of a fluorescence-based assay for serotonin has enabled us to visualize serotonin in non-mammalian blood cells. Immunohistochemical assays for serotonin have long been used in comparative phylogenetic studies of intestinal and neuronal tissues [13, 39]. However, our modifications of this method for blood cells offered a new optical test for blood serotonin in different species.

This study demonstrates for the first time that serotonin circulates in the thrombocytes of three reptilian species, the leatherback sea turtle, the green sea turtle and the American alligator. These findings were verified by quantitative HPLC measurements of serotonin. The leatherbacks are among the largest living reptiles and are capable of body temperature regulation [47, 48]. They can use large body size, circulatory changes, and peripheral insulation to maintain a warm body temperature in frigid waters and to avoid overheating in tropical waters. The vasoactive properties of serotonin may aid the leatherbacks in their thermoregulation.

Although occasional basking at the water surface, or on beaches, has been observed for some sea turtle species, this behavior does not occur frequently enough to represent a major source of incoming energy [49]. These observations have raised the possibility that green sea turtles are at least partially endothermic [50]. The regulatory mechanisms of heat exchange in sea turtles are still poorly understood. There is, however, evidence that they

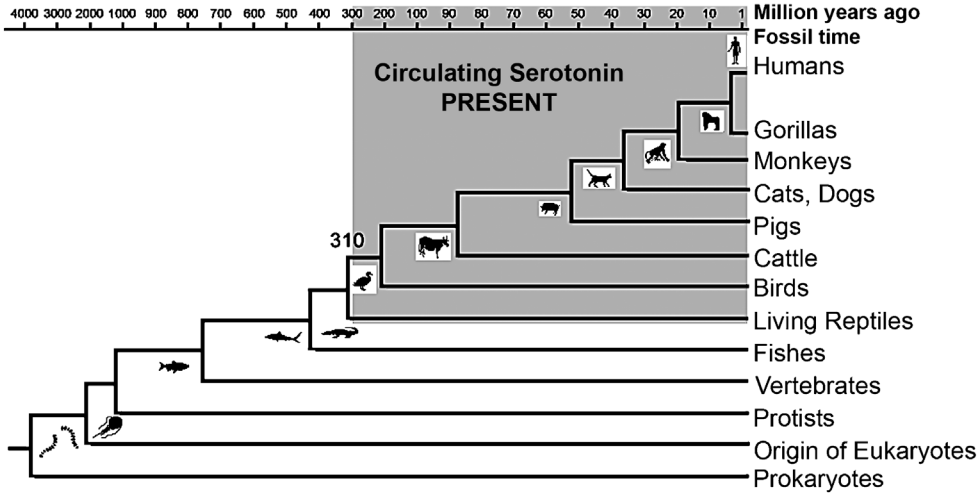

Figure 6. Phylogenetic comparison of species with and without circulating serotonin. Fossil records were used for the comparison of species divergence times. Time estimates are based on published studies [44, 69] and modified with permission. The timescale is only linear within the ranges 10–100, 100–1000 and 1000–5000 million years. The diversion of living reptiles versus mammals at 310 million years ago is a calibration point where fossil time and molecular clocks result in very similar estimates. Serotonin appears in the circulation in partially endothermic (American alligator) or truly endothermic (leatherback turtle) reptiles that evolved approximately 310 million years ago. Avian and mammalian blood also contains serotonin.

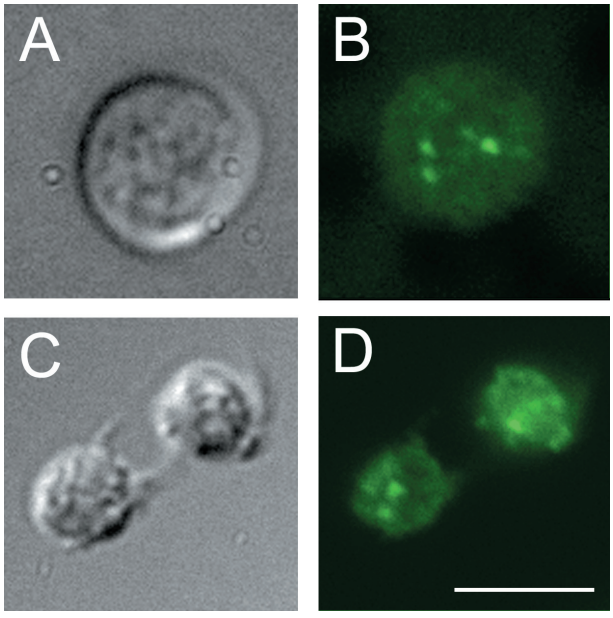

Figure 7. Effect of cooling on human platelet serotonin. DIC (*A*, *C*) and fluorescence (*B*, *D*) micrographs of human blood platelets fixed at 37 °C (*A, B*) and 20 °C (*C*, *D*) show that platelets are sensitive to low temperature. At 37 °C, platelets have a discoid shape, which is the prevalent morphology of platelets in the circulation, indicating that the cells are not activated. Serotonin-specific immunofluorescence detects serotonin localized in randomly distributed dense granules. Platelets change shape at low temperature, as seen by the contraction of the cell body with concomitant formation of pseudopodia (*C*), and uptake of serotonin (*D*). Scale bar, 5 µm.

heat up faster than they cool down, indicating the involvement of physiological control mechanisms. The front flippers, for example, possess both a large surface area over which heat can be exchanged and a relatively high resistance to blood flow. If this resistance were regulated by vasoconstriction and vasodilatation, then the circulatory system of the flippers would be an important thermoregulatory tool [51]. Interestingly, the red-ear slider, which is a small, cold-blooded fresh water turtle, does not have circulating serotonin.

Although American alligators show much higher variations of body temperature with changing environmental temperatures, they are capable of biochemical acclimatization. Acclimatization has been shown to be important in thermoregulation of these reptiles and behavioral patterns alone are not sufficient to explain their thermoregulatory abilities [52]. Again, serotonin contained in alligator thrombocytes might be involved in their partial endothermy. Bergmann's rule says that within a species, the body size increases with decreasing environmental temperature [53]. The hypothesis that peripheral serotonin appeared with endothermy does not conflict with this rule since serotonin also regulates food intake and obesity [54, 55]. Apparently, turtles and alligators follow this rule with their capability of gigantothermy, i.e., the maintenance of a constant, relatively high body temperature by having a large body and insulation. Large animals have a relatively low ratio of surface area to volume that allows them to retain heat better than smaller animals. Lizards and snakes do not use this strategy [53]. Since reptiles are viewed as intermediates in evolution [56], any biochemical marker that appears during reptilian evolution could be of potential interest for developmental changes.

Tuna are capable of maintaining a body temperature that is several degrees different from the environmental temperature. Thermoregulation in tuna was previously suggested to be based on changes in retial blood flow [57]. Retia are nets of blood vessels that can retain heat with exceptional efficiency [58]. How the absence of vasoactive circulating serotonin might support this view requires further investigation.

In the mammalian brain, serotonin regulates body temperature in addition to many other bodily functions. The vasoactive properties of serotonin in the periphery have been extensively studied and temperature effects of peripheral serotonin and serotonergic drugs have frequently been reported [59–62], but a general evolutionary link between serotonin and thermoregulation has not been considered. In general, homeothermy is costly because much energy is required to maintain the body temperature of an organism above ambient temperature. Most often, the ability to gather enough food is a critical factor for homeotherms. The selective factors that led to endothermy and homeothermy in mammals and birds have still not been identified [63]. The observation that an ocean-dwelling mammal such as the Steller sea lion transports high levels of serotonin inside platelets is fascinating in this context.

Serotonin is known to change skin perfusion [64] by contracting cutaneous arterioles in response to cooling [65, 66] and dilating cutaneous vessels in response to heat [8]. Human platelets change shape and take up serotonin when exposed to low temperature [46], which appears to contradict the notion that serotonin exerts its vasoactive properties by interacting with endothelial cells. Platelets might, however, facilitate this interaction, as they are pushed to the vessel wall by the red blood cells providing localized interaction between serotonin bound to the platelet surface and vascular endothelial cells. More research is required to determine the details of this interaction. Similar interactions have been reported for platelet-induced alterations of the chemotactic and adhesive properties of endothelial cells [67, 68].

In summary, this study showed for the first time that serotonin circulates in sea lion platelets and thrombocytes of sea turtles and the American alligator. The fact that serotonin was not found in the blood of the red-ear slider, a fresh water turtle, American bullfrog, Yellowfin tuna, and Chinook salmon suggests an evolutionary divergence about 310 million years ago or less. High levels of sero-

tonin in Steller sea lion platelets and the temperature sensitivity of human blood platelets provide additional support for the hypothesis that circulating serotonin might have emerged with endothermy but more experiments are needed to elucidate the significance of circulating serotonin for endothermy. Future studies might be directed towards identifying a possible role for serotonin in hibernating mammals, marsupials, and snakes. In vitro experiments measuring the interaction of serotonin with isolated blood vessels from different vertebrate species need to be performed to assess the species-specific vasoactivity of serotonin. In addition, differentiation of the *tph1* gene might complement the presence of serotonin in thrombocytes and could be used to map the divide between warm- and cold-blooded animals.

Acknowlwedgements. I thank the investigators D. Rosen, D. Whiteside, D. Jones, M. Hughes, A. Brown, M. Gardner, R. Brill and B. Reid, and W. Milsom, C. Sanders, and R. H. Devlin who provided blood samples or facilitated blood collection from Steller sea lions, elephants, duck, seagulls, sea turtles, American alligators, Yellowfin tuna, American bullfrogs, and Chinook salmon. I also thank M. V. Monsalve, R. H. Devlin and W. Milsom for their helpful comments that substantially improved the manuscript. This study was supported in part by Canadian Blood Services and the Canadian Institutes of Health Research (MOP-61828).

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