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Physiological Ischemia/Reperfusion Phenomena and Their Relation to Endogenous Melatonin Production

An Hypothesis

Dun-Xian Tan,[1] Lucien C. Manchester,[2] Rosa M. Sainz,[1] Juan C. Mayo,[1] Josefa León,[1] and Russel J. Reiter[1]

[1]*Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA; and* [2]*Department of Biology and Biochemistry, St Mary's University, TX, USA*

Ischemia/reperfusion is a frequently encountered phenomenon in organisms. Prolonged ischemia followed then by reperfusion results in severe oxidative injury in tissues and organs; however, some species can tolerate such events better than others. In nature, arousal from hibernation and resurfacing from diving causes animals to experience classic ischemia/reperfusion and, somehow, these animals cope well with the potential oxidative stress. It has been documented that during these physiological ischemia/reperfusion events, the activities of several antioxidant enzymes and the levels of some small-molecular-weight antioxidants become elevated. For example, the potent small-molecular-weight antioxidant melatonin often attains especially high levels during these physiological ischemia/reperfusion events including during arousal from hibernation or in the newborns during delivery. Highly elevated melatonin production during these physiological ischemia/reperfusion episodes exhibits several features. First, this high melatonin production is transient and fits well with the time schedule of the physiological ischemia/reperfusion period; therefore, it is not related to the normal endogenous melatonin rhythm. Yet, this transient peak protects the animals from destructive oxidative processes that occur during these transition periods. Second, these high levels of melatonin seem to derive from several organs since pinealectomy does not totally reduce circulating levels of this agent. Third, high melatonin production present at arousal from hibernation or in the newborns at birth does not appear to be controlled by light, i.e., it occurs both during the day and at night, and the amplitudes of elevated melatonin levels are equivalent at these times. The significance of these findings is discussed herein. Based on currently available data, we hypothesize that melatonin plays an important role in the physiological ischemia/reperfusion, i.e., as a member of antioxidant defense system, to protect against the potential oxidative injury induced by the physiological ischemia/reperfusion.

Key Words: Ischemia; reperfusion; oxidative stress; melatonin; hibernation; newborn; antioxidant.

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Author to whom all correspondence and reprint requests should be addressed: Russel J. Reiter, Department of Cellular and Structural Biology, University of Texas Health Science Center, Mail Code 7762, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA. E-mail: reiter@uthscsa.edu

Physiological Ischemia/Reperfusion and Melatonin Production

Oxygen is a suitable electron recipient during chemical reactions. Oxygen is abundant in the Earth's atmosphere and makes up almost 21% of the air we breath. Throughout evolution, the majority of organisms selected the use of oxygen for energy metabolism. Aerobic metabolism as a consequence of using oxygen as an ultimate electron acceptor in the electron respiratory chain generates more ATPs than are produced during anaerobic metabolism. Thus, oxygen is essential for aerobic organisms. In addition its obvious benefits, use of oxygen also exhibits unwanted side effects in organisms. Oxygen metabolism produces several reactive oxygen species (ROS) including the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), and singlet oxygen ($^1O_2$). Owing to highly reactive nature of ROS, these species readily attack the building materials of life including DNA, proteins, lipids, and carbohydrates to cause injury in all cells, organs, and organisms. The injury induced by ROS in organisms is referred to as oxidative damage or oxidative stress.

Fortunately, organisms have also evolved a complex antioxidant system to protect themselves against ROS. These include the antioxidant enzymes that metabolize ROS and repair enzymes to refurbish the macromolecular injury caused by ROS; the system also includes small molecular antioxidants that directly scavenge ROS. Under normal conditions, ROS levels and the antioxidant capacity are in delicate balance. The antioxidant defense system attempts to keep ROS levels in check. However, under certain conditions,

this balance favors the production of ROS. One of these conditions occurs in ischemia/reperfusion (anoxia/reoxygenation) injury.

Prolonged ischemia causes serious damage to tissues with early reperfusion being an absolute prerequisite for the survival of the ischemic tissue. However, reperfusion is a "double-edged sword" because reoxygenation often contributes to further tissue damage, i.e., reperfusion injury *(1)*.

The underlying mechanisms of ischemia/reperfusion injury are not entirely known, but existing evidence suggests that oxygen free radicals, generated during the first few minutes of reflow, cause extensive tissue injury *(2,3)*. Free-radical scavengers and antioxidants have been used successfully to protect against ischemia/reperfusion injury in cells, organs, and organisms *(4,5)*.

Melatonin (*N*-acetyl-5-methoxytryptamine) is the secretory product of the pineal gland in vertebrates *(6,7)* and exhibits several important physiological functions [for the extensive reviews see Reiter *(8,9)*]. Melatonin is one of the most conservative molecules during the course of evolution. It is present in virtually all organisms ranging from bacteria to primates [for an extensive review see Hardaland and Poeggeler *(10)*]. Melatonin was also found to be a potent endogenous free-radical scavenger in 1993 *(11)*. Since the discovery of melatonin as a free-radical scavenger, in excess of 1000 publications have directly or indirectly confirmed this finding. Recently, based on its all-round free-radical-scavenging capacity, Sofic et al. *(12)* referred to melatonin as a universal antioxidant. It detoxifies the $^{\bullet}OH$, H_2O_2, nitric oxide radical ($NO^{\bullet}$), peroxynitrite anion ($ONOO^-$), $^1O_2$, $O_2^{-\bullet}$, and peroxyl radical ($LOO^{\bullet}$) *(13,14)*. Melatonin exists in edible plants and herbal medicines in high levels and this plant-derived melatonin is consumed in the diet. Thus, melatonin is legitimately referred to as an antioxidant vitamin *(15)*. In its capacity as an antioxidant, accumulated data show unequivocally that melatonin effectively prevents ischemia/reperfusion injury in the retina *(16)*, brain *(17)*, liver *(18)*, stomach *(19)*, ileum *(20)*, fetus *(21)*, vasculature *(22)*, heart *(23–33)*, and other organs [for the extensive review see Reiter and Tan *(34)*]. Endothelial cells are an early target of ischemia/reperfusion damage and prevention of vascular destruction helps to preserve the tissue supplied by these vessels. Melatonin is highly effective in preventing vascular damage induced by ischemia/reperfusion at the microcirculatory level *(22)* (Fig. 1).

In addition to pathological ischemia/reperfusion which occurs during transient obstruction of blood flow or during a heart attack, there are some physiological ischemia/reperfusion phenomena in nature. These include arousal from hibernation, initiation of autonomic respiration in newborns, re-surfacing from diving, or during penile erection and detumescence. These events result in drastic changes in oxygen availability for tissues and organs and could produce substantial ischemia/reperfusion injury. However, a number of animal species are able to tolerate, under natural conditions,

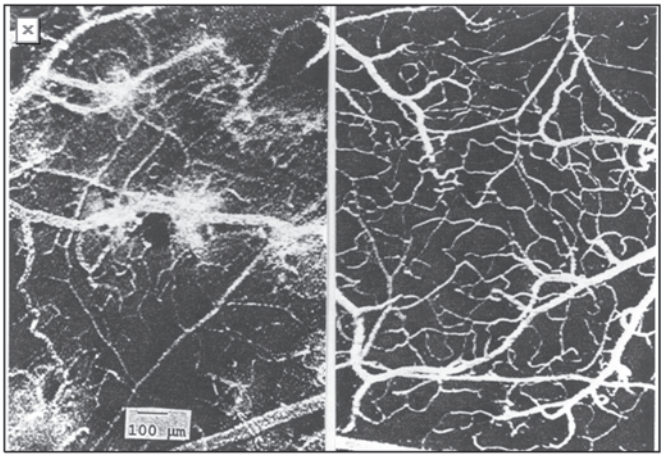

Fig. 1. Microvascular network after ischemia/reperfusion in a melatonin-treated hamster cheek pouch (right panel) and in a non-melatonin–treated animal. Melatonin prevents the increased permeability of the capilaries induced by ischemia/reperfusion and the vessels appear intact and capillary perfusion is preserved. The left panel is without melatonin treatment. An increase in vascular permeability with obvious edema is evident (modified from ref. *22*).

the ischemia/reperfusion situations mentioned above and protect themselves against potential oxidative damage induced by the anoxia/reoxygenation. As an example, Hermes-Lima and Storey *(35)* reported that there was no significant oxidative damage observed in a 30 h episode of anoxia in the leopard frog (*Rana pipiens)* followed by reoxygenation. A similar period of anoxia followed by reperfusion in other species would produce severe ischemia/reperfusion injury. High ischemia/reperfusion tolerance is also observed in several anoxia-tolerant organisms *(36)*. A strategy evolved to protect against natural ischemia/reperfusion insults in these organisms which was to increase their antioxidant capacity by elevating the antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione *S*-transferase, glutathione reductase) and antioxidant generation (e.g., glutathione) *(36)*.

Although melatonin, usually when exogenously supplied, is highly effective in protecting against pathological ischemia/reperfusion injury *(34,37)*, little is known as to whether this antioxidant participates in the defensive mechanisms against a physiological ischemia/reperfusion episode. Herein, we review some available data regarding the potential relationship of physiological ischemia/reperfusion and endogenous melatonin production.

Hibernation/Arousal and Melatonin Production

Hibernation is an adaptation of some animals to low environmental temperatures and reduced food availability. Hibernation is characterized by a global reduction in metabolism, body temperature, blood flow, and oxygen and energy generation and consumption while arousal from hibernation is achieved by the reversal of these processes. Melatonin lev-

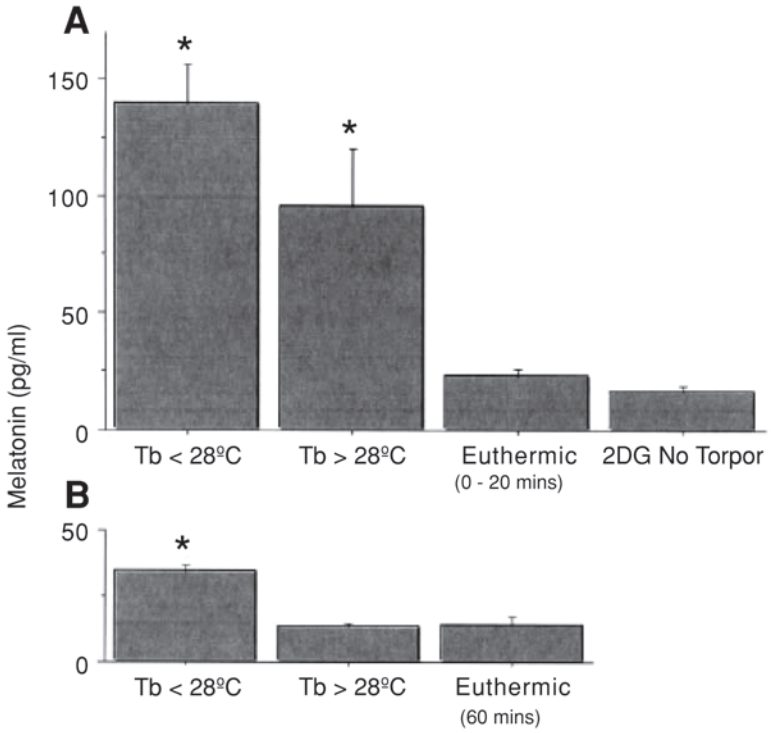

Fig. 2. Serum melatonin levels during arousal from torpor in hamsters injected with 2-deoxy-glucose (2-DG) 2 h after light onset and immediately transferred to darkness (**A**) or kept in the light (**B**). Melatonin levels were elevated during arousal from torpor in darkness compared with hamsters that did not undergo torpor. Tb, body temperature; $*p < 0.01$ compared with euthermic animals (modified from ref. *41*).

els during the transition from hibernation to arousal have been measured in several hibernating species.

Hamsters

Venecek et al. *(38,39)* initiated a study to investigate the relationship of pineal melatonin to hibernation or arousal in Syrian hamsters (*Mescocricetus auratus*). In their study, 30-d-old male hamsters were caged singly in a cold temperature ranging from 8 to 14°C. After 4 mo, the animals hibernating for the previous 2 d were transferred to warm temperature (28–30°C) at different times during the light–dark cycle and shaken to provoke arousal. During the period of hibernation, no melatonin rhythm was detected in the pineal gland of this animal, the golden hamster. However, during arousal, pineal melatonin levels rapidly increased regardless of whether hamsters were provoked to arousal during the night or during the day.

A similar phenomenon was also found in Turkish hamsters (*Mesocricetus brandti*) *(40)*. During the 4–5-mo hibernation season, Turkish hamsters are known to display 4 to 8 d of torpor (body temperature at 7–9°C) alternating with 1–3-d intervals of euthermia (body temperature 35–37°C). There was no detectable pineal melatonin during torpor. However, during arousal from a torporous bout, pineal melatonin in Turkish hamsters displayed increases no different in phase or amplitude from those in the pineal of animals held at an ambient temperature of 22°C.

In lieu of pineal melatonin, Larkin et al. *(41)* recently measured circulating melatonin levels during an episode of arousal from daily torpor in the Siberian hamster (*Phodopus sungorus*). The levels were estimated in hamsters experiencing arousal from natural daily torpor or torpor induced by 2-deoxy-D-glucose (2-DG) treatment (2500 mg/kg, intraperitoneal); it was found that arousal from deep hibernation, no matter if this is natural torpor or chemically induced torpor, is accompanied by a transient rise of melatonin in the blood. Especially during the early phase of arousal (body temperature <28°C), circulating melatonin levels are several-fold higher during arousal than they are during the euthermic phase. This large increase in melatonin is associated with the maximal period of tissue reperfusion by oxygenated blood. Even though hamsters kept in the light during the torporous bout reduce the magnitude of the melatonin rise related to arousal, the levels were still significantly elevated above basal values during the early arousal in animals treated with 2-DG (Fig. 2). These arousal-related elevations of circulating melatonin regress to basal values once the animals reach the euthermic phase from both natural daily torpor and torpor induced by 2-DG treatment.

Marmots

Two studies have investigated plasma melatonin levels of marmots (*Marmota flaviventris*) during their arousal from hibernation. In the first study, Florant et al. *(42)* observed

that during hibernation, plasma melatonin levels were low (<30 pg/mL) and without a rhythm, which is similar to the situation in hibernating hamsters. Upon arousal, once the body temperature reaches 23°C, plasma melatonin levels rapidly increased (80–130 pg/mL). In a follow-up study, the influence of light on the melatonin profile in this species during arousal from hibernation was investigated. After careful analysis of the data from this study, it is obvious that during arousal from the hibernation (the body temperature increased from 7 to 35°C within 2 h) circulating melatonin levels of the marmot are much higher (average 74 pg/mL, calculated from the data presented in Table 1 of their paper) than that in their normothermic, light-exposed counterparts (around 30 pg/mL estimated from the Figs. 1 and 2 of their paper). Melatonin levels in marmots are very sensitive to light exposure, with a light irradiance of 3.1 lx for 30 min at the animal's eye level suppressing circulating melatonin levels from about 80 to 30 pg/mL at night. Interestingly, during arousal, even during a 2-h normal room light exposure (light intensity about 500 lx and about 170-fold higher intensity than that which suppresses melatonin production) did not inhibit elevated melatonin production that accompanies arousal *(43)*.

Squirrels

The pineal melatonin content in a hibernating golden-mantled ground squirrel (*Spermophilus lateralis*) has been measured by Stanton et al. *(44)*. It was found that pineal melatonin levels were markedly reduced during a hibernation bout. Maximal suppression of the pineal melatonin content occurred late in the hibernation bout. In a follow-up study, this group *(45)* observed an increase in pineal melatonin content during the interbout; unfortunately, the blood melatonin concentrations were not been measured in this study although, as with pineal concentrations, it can safely be assumed that they were likewise elevated.

Recently, using a combination of *in situ* hybridization and immunohistochemical methodologies to evaluate the arylalkylamine-*N*-acetyltransferase (AA-NAT) mRNA expression in brains of both hibernating and non-hibernating thirteen-lined ground squirrels (*Spermophilus tridecemline-atus*), Yu et al. *(46)* observed that hibernating animals, but not non-hibernating squirrels, expressed significantly ($p < 0.01$) elevated levels of AA-NAT mRNA in both the epithalamic medial habenular nuclei (MHb) and in the hypothalamic suprachiasmatic nuclei (SCN). AA-NAT is often considered the rate-limiting enzyme in melatonin biosynthesis. This finding implies that the machinery for melatonin biosynthesis has been fortified extrapineally during hibernation, and that large amounts of melatonin, at least from these sites, may be available at the transition from hibernation to arousal. There is, unfortunately, little information available on blood melatonin levels of the ground squirrels during arousal. Judging the data from hamsters and marmots as well as the increased AA-NAT mRNA in ground squirrels, it can be predicted that an elevated blood melatonin level occurs during arousal in squirrels as in other species.

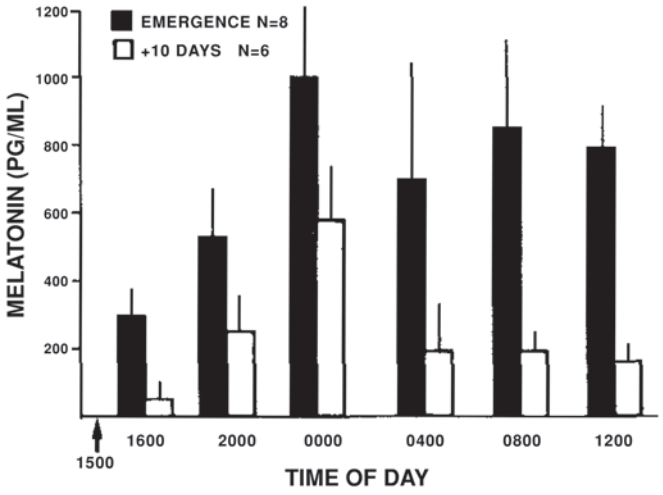

Fig. 3. The serum melatonin levels of garter snakes during the emergence and 10 d after emergence (modified from ref. *48*).

Snakes

The circadian rhythm of blood levels of melatonin in red-sided garter snakes (*Thamnophis sirtalis parietalis*) has been documented *(47)*. Red-sided garter snakes are usually in hibernation from late September to early May. Mendonca et al. *(48)* measured blood melatonin concentrations in this species during their hibernation period and upon their emergence. During hibernation, there is no detectable melatonin and no evidence of a melatonin rhythm was uncovered; upon emergence, however, blood melatonin levels rapidly increased from undetectable to about 900 pg/mL within 1 h during the photophase (Fig. 3). The levels of melatonin during emergence are about 20 times higher than those that are considered normal blood melatonin concentrations in the garter snake during the photophase (about 40 pg/mL) *(47)*. The high circulating melatonin levels are maintained for up to 24 h after the snake's emergence. Ten days following emergence, a diel cycle of melatonin production is re-established and the circulating melatonin levels revert to normal values. Pinealectomy in garter snakes before hibernation reduces plasma melatonin levels; however, upon emergence from hibernation, both pinealectomized and pineal-intact garter snakes exhibit very high circulating melatonin levels. These surges in melatonin production are phenomena common to both pinealectomized or pineal-intact garter snakes, and the melatonin levels are equivalent in these two groups *(49)*. The difference is that within 10 d after emergence, the pineal-intact snakes display a normal diel cycle of melatonin production while the pinealectomized snakes exhibit no such rhythm. The results indicate that pineal melatonin generation is the source of circulating melatonin rhythm, while the melatonin surge upon emergence from hibernation is independent of the pineal gland.

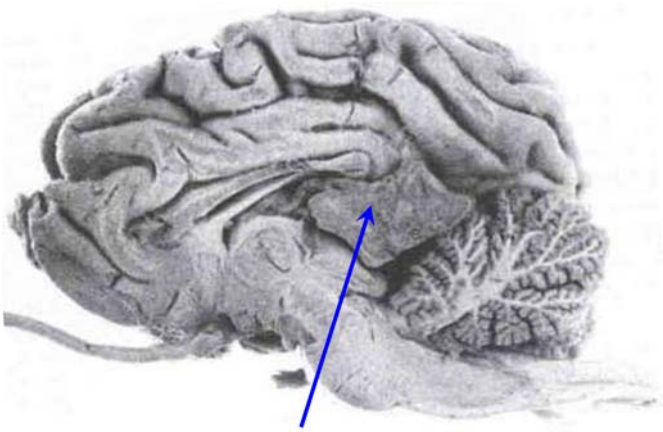

Fig. 4. The huge pineal gland of a newborn Weddell seal is indicated with an arrow (modified from ref. *50*). The pineal gland of seals is generally larger than this organ in other species.

Table 1
Summary of Information Relating to Melatonin Levels in Newborn Seals[a]

Strains	*n*	Postpartum days	Pineal weight (mg)	Pineal melatonin content (ng)	Plasma melatonin (peak) (pg/mL)
Southern elephant seal *(Mirounga leonina) (52)*	37	0–1	4710 ± 350		4091 ± 1328
Northern elephant seal (*Mirounga angustirostris*) *(53)*	4	0–5	3000 ± 800		700–2320
Harp seal (*Phoca groenlandica*) *(54)*	11	0–14	273 ± 45	49 (Median)	3500–5500*
Grey seal (*Halichoerus grypus*) *(54)*	6	4–10	337 ± 74	90 (Median)	6800–7200*
Hooded seal (*Cystophora cristata*) *(54)*	2	0–14	520–1289	254–7600	4500–6500*
Weddel seal (*Leptonychotes weddelli*) *(51)*	2	0–23	3500–3600	>2000	

[a]The reference number is listed in parentheses after the species name.
*Estimated from the figures in the cited references.

Newborns and Melatonin Production

From delivery to the onset of autonomic respiration, newborns experience physiological ischemia/reperfusion. Circulating melatonin levels in the pregnant mother near term and in the umbilical cord blood as well as in the blood of newborns have been investigated in animals and humans.

Seals

The seal is one of few species in which the pineal gland and blood melatonin levels of newborns immediately after parturition have been studied. It is documented that the largest pineal gland found in any species is that of the newborn Weddell seal (*Leptonychotes weddelli*) *(50,51)*. The pineal gland in the newborn seal occupies a considerable portion of their intracranial cavity (Fig. 4). The authors speculated that the large pineal gland in newborn Weddell seals is essential for them to adapt to harsh Antarctic environments. The large gland typical of newborn Weddell seals gradually regresses with increased age.

Little and Bryden *(52)* measured plasma melatonin levels in newborn southern elephant seals (*Mirounga leonina*). They found that the plasma melatonin in newborns is extremely high. Mean plasma melatonin levels during the 0–24 h interval post-partum is 4091 ± 1328 pg/mL (compared to < 40 pg/mL in adult). This extremely elevated newborn plasma melatonin is also accompanied with an huge pineal gland, which has an average weight 4.71 ± 0.35 g (the pineal gland in adult human is about 0.15 g). Very high plasma melatonin levels have also been observed in the northern elephant seal (*Mirounga angustirostris*) immediately after delivery *(53)*. The midday levels of plasma melatonin in newborn northern elephant seals average more than 700 pg/mL and during night the levels increase to 2320 pg/mL for the first 5 d post-partum and then declined rapidly to less than 93 pg/mL after d 9. A similar phenomenon of high levels of pineal and blood melatonin has been verified in different strains of newborn seals including harp seals (*Phoca groenlandica*), grey seals (*Halichoerus grypus*), and hooded seals (*Cystophora cristata*) *(54)*. Their pineal and circulating melatonin levels are enumerated in Table 1. The usual tendency is that highest plasma melatonin levels appear during the first post partum day.

Humans

It is difficult to obtain blood samples for measuring circulating melatonin levels in human newborns immediately

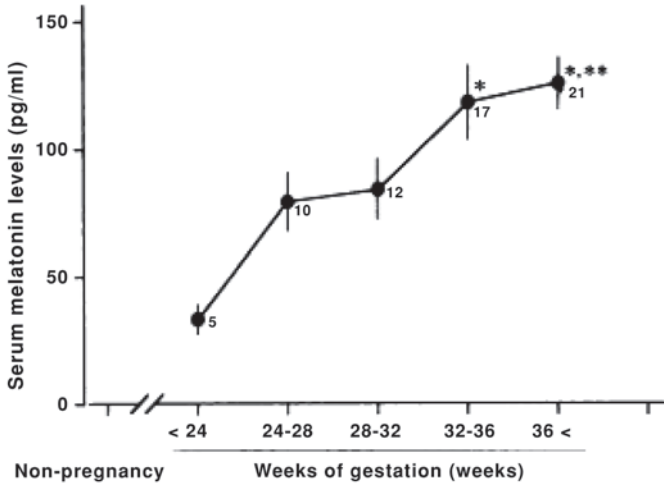

Fig. 5. Changes in serum melatonin levels at night in pregnant women (modified from ref. *58*).

after parturition. However, in lieu of directly measuring melatonin in the blood of newborns, alternative methods can be used to estimate melatonin levels in newborns, including the measurement of the mother's plasma melatonin at full term because melatonin is readily transported to fetus through the placenta *(55)*; also, plasma melatonin levels have been measured in umbilical cord blood immediately after delivery. Pang et al. *(56)* first observed that melatonin levels in pregnant women exhibit two phases. Plasma melatonin is reportedly high during the first 20 wk of gestation and then the levels drop until 33 wk; thereafter, melatonin values increase again at near full term. The high plasma melatonin levels at delivery of the newborn rapidly decreased 1–5 min post-partum. Acuna et al. *(57)* noted that during pregnancy, blood melatonin levels increased from 28 pg/mL in the first 3 mo to 70 pg/mL at delivery. Nakamura et al. *(58)* found that beginning at 22 wk of gestation, plasma melatonin levels of pregnant women gradually increased until parturition (Fig. 5). These elevated melatonin levels rapidly dropped after delivery. Studies have uniformly shown increased plasma melatonin levels in pregnant full-term women. The high levels of circulating melatonin in pregnant women at full term are attributed to both mother and fetus *(58)*.

In addition to the mother's blood, rather high levels of melatonin are present in amniotic fluid during delivery (262 ± 22 pg/mL) as reported by Mitchell et al. *(59)*. As to melatonin levels in umbilical cord blood, the results are also relatively consistent. Pang et al. *(56)* claimed that melatonin levels in umbilical cord blood were lower than that in mother's blood. The absolute melatonin level in umbilical cord 1–5 min post-partum was reported to be 20 ± 2.9 pg/mL, which is only slightly higher than basal levels in adult humans during the day. Several other studies, however, have identified highly elevated melatonin values in umbilical cord blood. Lang et al. *(60)* reported the melatonin concentrations of 70.6 ± 10.9 and 57.9 ± 10.9 pg/mL in umbilical artery and vein, respectively, and Mitchell et al. *(61)* observed similar levels of umbilical cord blood melatonin with the arterial level being 65 ± 8 pg/mL and venous level being 82 ± 9 pg/mL. Vicente et al. *(62)* found that the melatonin levels in serum obtained from umbilical cord blood of full-term human neonates at the time of delivery are in excess of 100 pg/mL, and these high levels of melatonin are very consistent regardless of whether the delivery occurs during the day or at night. Thus, light exposure does not suppress high levels of melatonin production in umbilical cord as it normally does.

Similar results have been obtained when direct measures of melatonin in the blood of newborns were performed. Munoz-Hoyos et al. *(63)* measured circulating melatonin levels during the first 72 h of life in human infants. The values during the day (09:00–21:00 h) of 119 newborns were 147 ± 24 pg/mL with nighttime melatonin levels being 146 ± 32 pg/mL. These day time values are sevenfold higher than would normally be present in adult human during the day. Also, the similar levels of melatonin during the day and night imply that even during the day melatonin production is high and obviously not suppressed by light.

Sheep

Kennaway and Seamark *(64)* measured the activity hydroxyindole-*O*-methyltransferase (HIOMT), a key enzyme of melatonin biosynthesis, in the pineal gland of fetal sheep. They observed that, 2 d before parturition, HIOMT activity in the pineal gland of fetal sheep was unexpectedly elevated. They surmised the increase in pineal HIOMT and the presumptive release of melatonin was obligatory for the parturition process. In a follow-up study, they *(65)* confirmed that the elevated HIOMT activity in fetal pineal is observed only in the final days of gestation. Coincidentally, an accumulation of melatonin in the fetal sheep pineal was also observed, but there was no clear correlation between pineal melatonin and the plasma melatonin levels in the fetus during the last few days of gestation.

The Potential Relations of Diving and Melatonin Production

Diving, especially when it is prolonged, is a classic physiological ischemia/reperfusion. There are no indications on melatonin alternations before, during, or after diving owing to the technical difficulties with blood collection. All data concerning the relation of diving to melatonin production are indirect. This indirect information, however, may provide us some idea as to whether high levels of melatonin are present in this event. Generally, some diving animals indeed possess a relatively large pineal gland such as in seals, sea lions, and walruses *(66)*. Aarseth et al. *(67)* have speculated that the large pineal gland and high melatonin levels in the feto-seals are temporary consequences of a fetal strategy to affect the maternal blood supply during diving.

In the follow-up study *(68)*, this group found that melatonin reduces noradrenaline-induced vasoconstriction in the uterine artery of pregnant hooded seals; thus, it seems that a large amount of melatonin synthesized by the fetus is involved in upholding maternal uterine blood flow during diving and protecting against the potential ischemia/reperfusion injury of the fetus. Griffiths et al. *(69)* observed that the timing of the diurnal and seasonal maxima in duration of diving is consistent with expected daily and annual peaks in melatonin levels in southern elephant seals. The data imply that the high levels of blood melatonin may be required for the prolonged diving in seals. In the study of dives of southern elephant seals, Bennett et al. *(70)* concluded that many of the tissues of southern elephant seals might be flooded by high concentrations of free radicals at the termination of each dive. It is possible that melatonin provides the tissues of southern elephant seals with extremely effective protection against reperfusion injury at the termination of diving. Melatonin therefore seems likely to be an important agent in mammalian divers *(70)*.

Erection and Melatonin Production

Erection also represents a physiological ischemia/reperfusion. Priapism has been proven to cause ischemia/reperfusion injury in penile tissues *(71–73)*. In the management of ischemic priapism, reperfusion causes erectile tissue injury owing to the presence of ROS. Although reoxygenation of the corpora cavernosa is obviously necessary for the recovery of normal function, it is associated with oxidative damage to the tissue. Melatonin has been reported to reduce the corpora cavernosa ischemia/reperfusion injury in rats *(74)*. There has not been, however, any attempt to measure the levels of melatonin in the blood of corpora cavernosa of the penis when erection occurs. We speculate that during an erection melatonin levels in the blood contained in the penis may be elevated by the local biosynthesis of melatonin. Many tissues and organs including testis *(75)* may be capable of producing melatonin. That potentially high local melatonin levels occur during an erection cannot be ruled out, and these elevated concentrations of melatonin may protect erectile tissues from damage caused by frequently encountered sexual events.

Disussion

Ischemia/reperfusion generates ROS and results in oxidative stress. Under most conditions, ischemia/reperfusion causes pathological alternations in tissues and organs, i.e., ischemia/reperfusion injury. Some ischemia/reperfusion events, however, are well tolerated by organisms under natural conditions. Hibernation/arousal, diving/resurfacing, erection/detumescence, and the onset of automatic respiration in newborns are reminiscent of ischemia/reperfusion events that are highly damaging under most conditions. For example, during arousal from the hibernation in the hamster, the blood supply to tissues that are rapidly warmed is increased in excess of 400% and the oxygen consumption increases 24.6 times *(76)*. Abrupt changes in blood re-supply and re-oxygenation in organs of arousing animals will inevitably generate large quantities of oxygen free radicals, especially in the brain. The brain is one of the first organs to which the blood supply is re-established during arousal from hibernation. Hibernating species, however, somehow easily adapt to this condition. By comparing the responses to this natural physiological ischemia/reperfusion, the most commonly observed response is an enhancement of antioxidant defenses. The increase in the basal activities of key antioxidant enzymes, as well as "secondary" enzymatic defenses, and/or glutathione levels in preparation for a putative oxidative stressful situation arising from tissue reoxygenation, may be essential for organisms to tolerate this physiological hypoxia and reoxygenation *(77)*. Indeed, increased activities of antioxidant enzymes, SOD, catalase, and glutathione peroxidase have been reported in the garter snake and other animals that do tolerate ischemia/reperfusion *(36,78)*.

Coincidently, melatonin, a potent endogenous antioxidant, also rises to high levels (compared to the normal physiological levels) during some of these physiological ischemia/reperfusion events; this is the case during arousal from hibernation and in full-term pregnant mothers and newborns. Evidence also indicates that high level of melatonin may also be present during diving in some species, which also seem to have an extraordinarily large pineal gland. This also indicates, at least in the garter snake, that the high levels of melatonin in their blood is not solely of pineal origin at the time of emergence from hibernation. It seems that the elevated melatonin levels during arousal have little to do with the thermo-regulation *(41)* or elevated adrenaline levels *(43)*. Moreover, high melatonin production during arousal or in newborns appears not to be influenced by light exposure; thus, this melatonin is not a signal of light and darkness as it is under normal conditions. Therefore, we hypothesize that the high levels of melatonin occur during physiological ischemia/reperfusion as part of an array of elevated antioxidant defenses to combat tissue and organ damage that would normally occur as a result of ischemia/reperfusion. Melatonin as an antioxidant has been proven to be very effective in reducing pathological ischemia/reperfusion injury in numerous experimental conditions *(37)*. In addition to exogenously supplied melatonin, studies have shown that physiological levels of melatonin also effectively reduce organ oxidative damage induced by ischemia/reperfusion *(79–81)*.

As already mentioned, elevated melatonin levels during physiological ischemia/reperfusion events such as during arousal from hibernation or at the time of birth appear not to be exclusively pineal related. Thus, pinealectomy does not suppress the high level of blood melatonin in garter snakes during their emergence from hibernation *(49)*. In addition, light exposure neither inhibits elevated melatonin

levels of hamsters during their arousal from hibernation *(39)* nor does it suppress high levels of umbilical cord blood or circulating melatonin in human newborns *(62,63)*. Conventionally, pineal melatonin production is inhibited by light with even short-term light exposure quickly suppressing pineal melatonin production *(82)*. The extra-pineal melatonin possesses an advantage over the pineal-derived melatonin in terms of protecting against physiological ischemia/reperfusion owing to the fact that it is not inhibited by light. This advantage allows for high levels of circulating melatonin during physiological ischemia/reperfusion events regardless if it is occurs during the day or night and it protects against potential oxidative injury.

It is not clear what mechanisms are employed by these organisms to elevate melatonin production during the episodes of physiological ischemia/reperfusion. It may be that organisms have already upregulated the gene expression of the key enzyme for melatonin biosynthesis in non-pineal sites at least during hibernation *(46)*. This preparative action in advance of arousal would make melatonin available during the forthcoming period of oxidative stress. The evidence is certainly consistent that during a hibernation bout, melatonin levels both in the pineal and in the blood are low. An obvious question that remains is how the up-regulation of gene expression for the enzymes required for melatonin biosynthesis occurs along with low melatonin production during a hibernation bout. It is likely that during hibernation when body temperature is roughly at 5–8°C, which is not optimal for enzymes to synthesize melatonin. Although the gene expression of AA-NAT is elevated, both enzyme activities and melatonin production are low. When the body temperature increases to the optimal reactive condition for AA-NAT during arousal, the activities of enzymes are rapidly increased, supported by the accumulated AA-NAT; this results in a rapid rise in melatonin as the organisms continue to warm.

In all situations described in this report, melatonin production is only transiently elevated. This transiently elevated melatonin production fits well with the transition from hypoxia to reoxygenation and the associated high free-radical production. These events support our hypothesis that the high level of melatonin present during physiological ischemia/reperfusion events functionally protects against the potential oxidative damage that would normally occur at these critical times. The transiently high levels of melatonin would also not disturb the normal melatonin circadian rhythm after the animals have transited to normal conditions (relative to the physiological ischemia/reperfusion). We also speculate that during an erection, high penile blood melatonin levels exist and the elevated melatonin concentrations in the blood of diving mammals would also be advantageous. To prove this hypothesis, carefully designed studies are warranted.

The high levels of melatonin present during physiological ischemia/reperfusion may be a complementary mechanism to increased antioxidative enzyme activity to protect against the elevated oxidative stress that would be a normal consequence of physiological ischemia/reperfusion. It has been reported that physiological ischemia/reperfusion is also associated with rises in other small-molecular-weight antioxidants including vitamin C, glutathione, and uric acid *(83–85)*. Small-molecular-weight antioxidants are very important members of the antioxidant defensive system that protect against injury resulting from hypoxia/reoxygenation that occurs under physiological circumstances. This is particularly important for those radical products, e.g., the $^{\bullet}OH$, that cannot be detoxified by antioxidant enzymes. Melatonin as a small antioxidant is very effective in scavenging this most destructive reactive agent.

In addition to melatonin, its metabolites including cyclic 3-hydroxymelatonin, N^1- acetyl-N^2-formyl-5-methoxykynuramine (AFMK), and *N*-acetyl-5-methoxykynuramine (AMK), which are generated during melatonin's reaction with ROS, also exhibit high antioxidant capacity *(14,86, 87)*. This unique property of melatonin and its metabolites magnifies its free-radical-scavenging capability. High levels of melatonin and its metabolites during physiological ischemia/reperfusion including hibernation/arousal, diving/resufacing, erection/detumescence, and delivery of newborns assist these organisms in coping with the potentially destructive transition period and reduce oxidative damage induced by these physiological ischemia/reperfusion events.

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