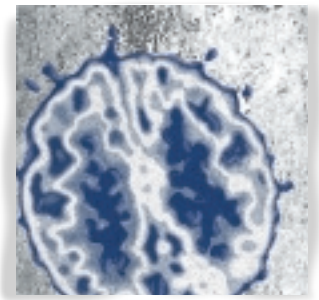
Role of melatonin in the induction and maintenance of sleep

Jean B. Fourtillan, PhD



Pharmacokinetic studies of melatonin in young and elderly human volunteers, and the measurement of hypnotic effects in chicks under alternate light–dark or permanent light conditions, show that melatonin is a bioprecursor of hypnotic acetyl metabolites produced by the enzymatic acetylation of both melatonin and 2-oxomelatonin under the control of serotonin N-*acetyltransferases (NATs), which are present in the pineal gland. The acetyl metabolite of melatonin, which we call carbo2, is an* N-*acetyl-β-carboline. The electroencephalographic (EEG) architecture of the sleep produced by this compound is similar to that of physiological sleep, and is characterized by the significant proportion of slow-wave deep sleep and rapid eye movement sleep. This is in sharp contrast to the EEG sleep architecture observed with GABAergic (GABA, γ-aminobutyric acid) compounds. Since insomnia and sleep disorders are believed to be due to a lack of NAT enzymes in the pineal gland, a new therapeutic approach of sleep disorders by administration of such hypnotic acetyl metabolites of melatonin, or synthetic analogs thereof, can be envisaged.*

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In higher vertebrates that are active during the day (eg, humans, chicks, and dogs, but not rats, which are nocturnal), nighttime melatonin secretion is temporally associated with sleep. Analysis of 24-h urine samples from young and elderly people alike *(Figure 1)*, with or without insomnia, clearly shows a direct correlation between sleep and urinary excretion of 6-sulfatoxymelatonin.[1] Subjects with insomnia have a considerably reduced production of melatonin from their pineal gland, which is due to a decrease in the level of the enzyme serotonin *N*-acetyltransferase (NAT). Insomnia could therefore be due to a lack of this NAT enzyme in the pineal gland.

These observations have led several groups to propose treating sleep disorders by administration of melatonin or melatoninergic compounds, in order to compensate for the lack of melatonin observed in subjects with insomnia.

Pineal melatonin secretion in humans

We have demonstrated that melatonin is a bioprecursor of hypnotic acetyl metabolites produced by enzymatic acetylation of melatonin and 2-oxomelatonin under the control of acetyltransferases, most probably the NAT enzymes.

In 1994, in our laboratory, we developed a specific and highly sensitive gas chromatography–mass spectrometry (GC-MS) method[2] to assay, simultaneously and distinctly, plasma concentrations of endogenous melatonin (D_0-melatonin) and exogenous melatonin (D_7-melatonin), in which 7 atoms of H have been substituted by 7 atoms of deuterium. Using the same human volunteers (12 young subjects in June 1994 and 12 elderly subjects in October 1994), we determined the pharmacokinetics of exogenous

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D_7-melatonin, when given orally and intravenously, and the kinetics of the pineal secretion of endogenous D_0-melatonin.[3,4]

The results shown in *Figure 2* led to the following conclusions:

- Secretion of melatonin by the pineal gland occurs only during the night.
- Pharmacokinetic analysis shows that the rate of melatonin secretion by the pineal gland is constant throughout the whole nocturnal pineal melatonin production, for the same subject.
- The clock times at the beginning and end of melatonin secretion from the pineal gland are the same for each subject, whatever the season and night length. Duration of melatonin pineal secretion is between 7.5 and 8 h. Therefore, melatonin secretion and sleep are contemporaneous.
- There is a large interindividual variability in the amount of melatonin released in plasma by the pineal gland during the night in young and old subjects alike.

Hypnotic effect of melatonin and NAT in the CNS

Results of previous related studies show that melatonin secretion, and therefore the presence of melatonin in the central nervous system (CNS), is necessary for the induction and maintenance of nocturnal sleep. However, the presence of melatonin in the CNS is insufficient for the induction and maintenance of sleep. Indeed, *Figure 3* and *Table I* show results of observations in chicks in an alternate light (L)–dark (D) program (L/D, 12 h:12 h), in which the light phase lasted from 8.00 AM until 8.00 PM. When melatonin was administered intramuscularly (pec-

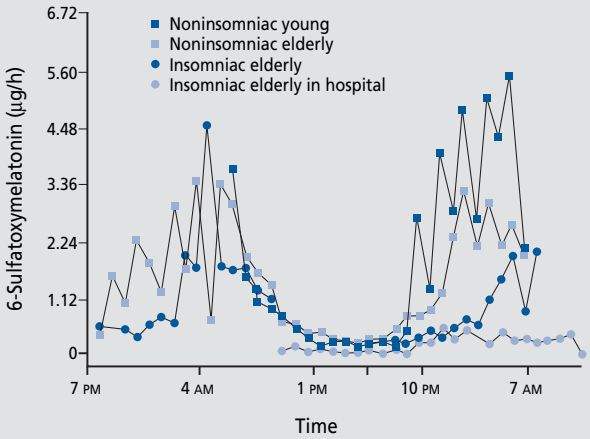
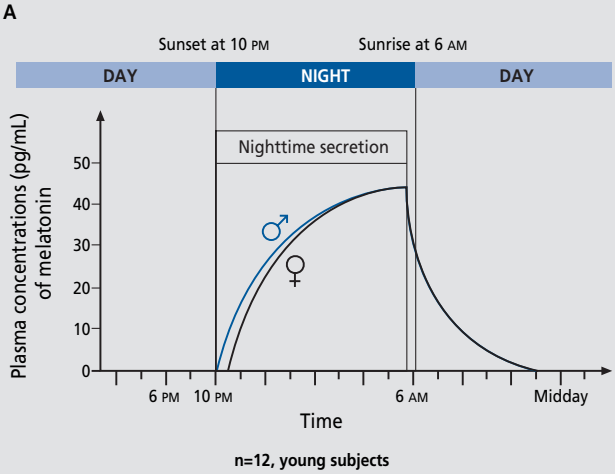


Figure 1. Mean 6-sulfatoxymelatonin concentrations over 36 h in young people without sleep disorders (dark blue squares), elderly people without sleep disorders (light blue squares), independently living elderly patients with insomnia (dark blue circles), elderly patients with insomnia living in nursing home (light blue circles).



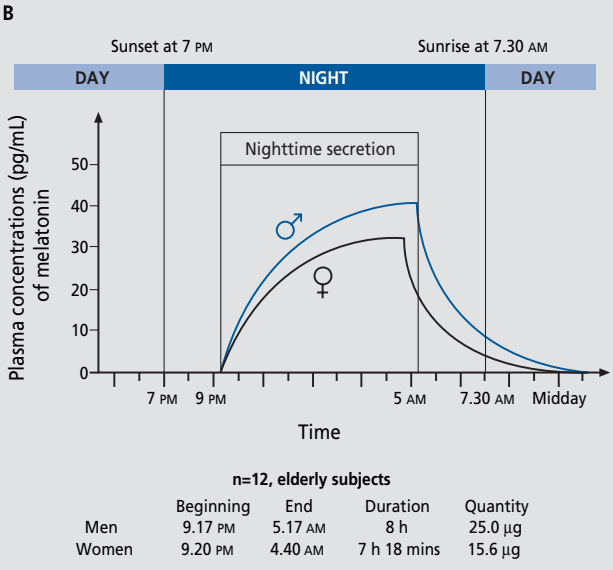


Figure 2. Nighttime plasma concentrations of melatonin in 12 young subjects (**A**: June 1994) and 12 elderly subjects (**B**: October 1994).

toralis major muscle) during the light phase from 2.00 PM to 8.00 PM, the chicks did not exhibit any signs of a hypnotic effect. The absence of a hypnotic effect during the light phase correlated with the very low level of NAT activity in the pineal glands of chicks measured at the same times.

In contrast, when chicks were observed in a 7-day permanent light program (L/L, 12 h:12 h), during which NAT activity level was constantly higher,[5] the administration of melatonin induced a significant hypnotic effect. The duration of sleep (between 4 and 5 h) was much greater than that observed with diazepam (between 1 and 2 h) when it was administered intramuscularly at the same dose (1 µM per 100 g body weight, at 2.00 PM).

	LD (low)	LL (high)
Placebo		
• Doze time (min)	0	0
• Sleep time (min)	0	0
Melatonin		
• Doze time (min)	0	4 to 5
• Sleep time (min)	0	240 to 300
Diazepam		
• Doze time (min)	4 to 5	4 to 5
• Sleep time (min)	30 to 40	50 to 117

Table I. Intramuscular (pectoralis major muscle) administration of melatonin, diazepam, and placebo in chicks under a 7-day alternate light–dark program (LD) (light 8.00 AM to 8.00 PM; dark 8.00 PM to 8.00 AM) or a permanent light program (LL). At 2 PM, the chicks were administered doses equivalent to 1 µM for 100 g body weight, dissolved in 0.2 mL of an ethanol–water mixture, 50/50, V/V.

These results lead to the following conclusions:

- The simultaneous presence of melatonin and NAT in the CNS (pineal gland) is a necessary and sufficient condition for the induction and maintenance of sleep.
- In contrast to the classic so-called "hypnotic drugs" (eg, benzodiazepines, barbiturates, zopiclone, and zolpidem), melatonin does not have direct hypnotic properties related to its chemical structure. Its hypnotic effects depend on the activity of NAT in the CNS.

Melatonin: a bioprecursor of hypnotic metabolites

During the development of the GC-MS method for the assay of melatonin in plasma,[2] our attention was focussed on the chemical reactivity of melatonin at position 3, which allows cyclization of the side chain after acylation. This proceeds by nucleophilic attack and leads to a fluoroacyl-β-carboline *(Figure 4)*.

Considering our previous observations, we assumed that melatonin undergoes enzymatic acetylation during the night, under the control of NAT, and that this leads to an *N*-acetyl-β-carboline, which we call carbo2. We conclude that melatonin is a bioprecursor of hypnotic acetyl metabolites, such as carbo2. We have validated this assumption in several ways.

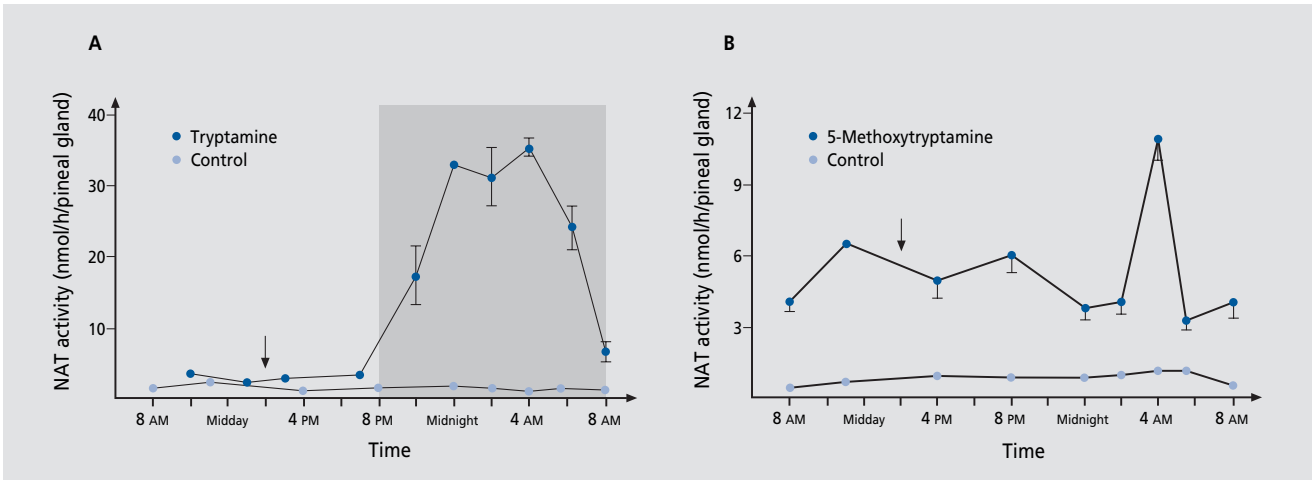


Figure 3. Change in *N*-acetyltransferase (NAT) activity in the pineal glands of chicks. **A.** Intramuscular (pectoralis major muscle) administration of tryptamine at 2 PM (arrow) in chicks in a 7-day alternate light–dark program (light 8.00 AM to 8.00 PM; dark 8.00 PM to 8.00 AM). The NAT activity increases from 8.00 PM and decreases from 4.00 AM. **B.** Intramuscular (pectoralis major muscle) administration of 5-methoxytryptamine at 2 PM (arrow) in chicks in a 7-day permanent light program.

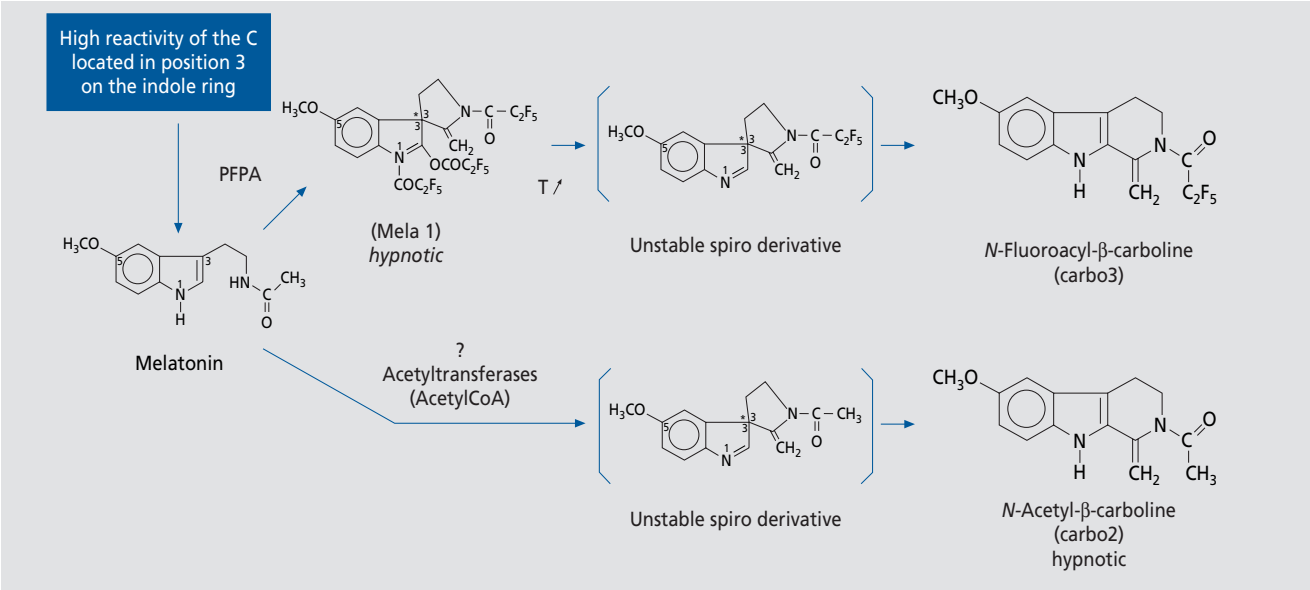


Figure 4. Perfluoroacylation of melatonin. Chemical structure of the fluoroacyl derivative obtained during the derivatization of melatonin using PFPA (pentafluoropropionic anhydride), according the gas chromatography–mass spectrometry (GC-MS) analysis. Acetyl CoA, acetyl coenzyme A; T, temperature.

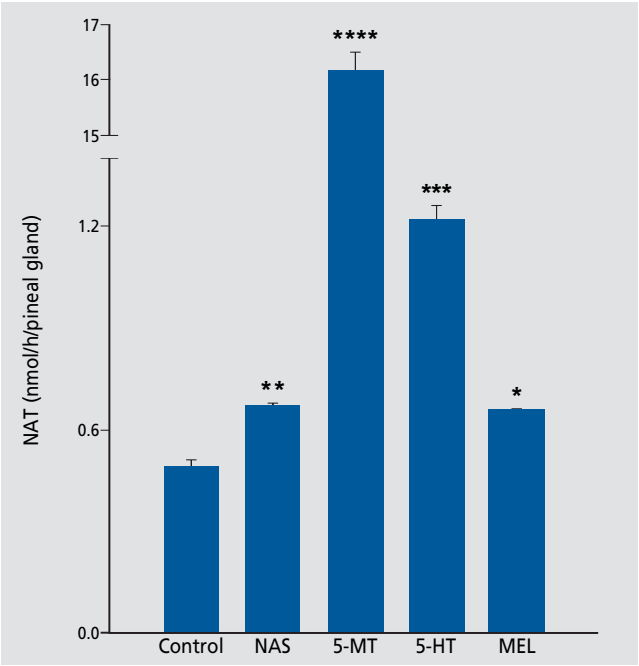


Figure 5. Acetylation capacity of various substrates in chick pineal glands in an alternate light–dark program (light 6.00 AM to 6.00 PM; dark 6.00 PM to 6.00 AM) and collected in the middle of dark phase (midnight). NAS, *N*-acetylserotonin; 5-MT, 5-methoxytryptamine; 5-HT, 5-hydroxytryptamine (serotonin); MEL, melatonin. *P<0.002; **P<0.001; ***P<0.0005; ****P<0.00005.

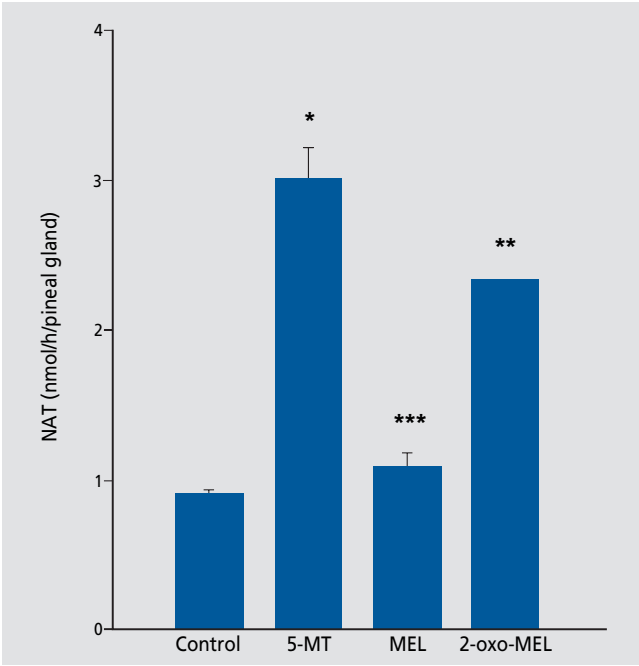


Figure 6. Acetylation capacity of various substrates in chick pineal glands in an alternate light–dark program (light 8.00 AM to 8.00 PM; dark 8.00 PM to 8.00 AM) and collected 1 h before the light phase (7.00 AM). 5-MT, 5-methoxytryptamine; MEL, melatonin; 2-oxo-MEL, 2-oxomelatonin. *P<0.0002; **P<0.00005; ***P<0.0005.

Acetylation of melatonin in chick pineal glands

Chick pineal glands were observed during an alternate light–dark program at 37°C for 7 days. In the middle of dark phase, they were treated with [^{3}H]acetyl coenzyme A and melatonin (or 2-oxomelatonin) for 30 min.

- *Figures 5* and *6* show that melatonin (or 2-oxomelatonin) undergoes an acetylation that is significantly higher (P<0.002, in the middle of dark phase; P<0.0005, 1 h before end of dark phase [or P<0.00005 for 2-oxomelatonin over the whole dark phase]) than that observed in controls (nonsignificant when melatonin was replaced by phosphate buffer).
- GC-MS indicated the biosynthesis of [^{3}H]carbo2 for five chick pineal glands collected in the middle of dark phase *(Table II)*.

Synthesis of carbo2

We have synthesized several acetyl derivatives, such as carbo2 *(Figure 7)*, which is an *N*-acetyl-β-carboline. We have found 20 to 40 pg carbo2 per gram of lamb pineal gland collected on the middle of the dark phase of an alternate light–dark program.

Hypnotic activity of carbo2

The hypnotic activity of carbo2 has been observed and measured in chicks and beagles:

- In chicks, the tests were performed at 2.00 PM, in the middle of light phase, a time at which NAT activity in the pineal gland is very low. The results are presented in *Table III*, together with some reference compounds. The essential role of acetyl group is demonstrated by the fact that 10-methoxyharmalan (as well as harmaline), which is the product of *N*-deacetylation of compound carbo2, does not exhibit any hypnotic effect. In contrast, it induces excitatory effects in chicks by increasing locomotor activity.

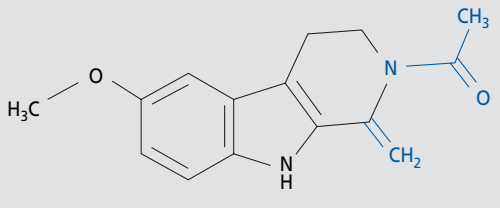


Figure 7. Molecular structure of carbo2 (*N*-acetyl-β-carboline).

- In beagles, polysomnographic studies showed that when carbo2 was administered intravenously, it induced sleep of longer duration and shorter time latencies than the sleep induced by zolpidem and diazepam *(Table IV)*.

The most interesting feature, which provides more support for our assumption, is the EEG architecture of the sleep produced, which is similar to that of physiological sleep (see results with placebo in *Table IV*), characterized by the significant proportion of slow-wave deep sleep and rapid eye movement (REM) sleep, in sharp contrast to the EEG sleep architecture observed with GABAergic (GABA, γ-aminobutyric acid) compounds, such as zolpidem or diazepam, which induce mainly drowsiness (light sleep) and little REM sleep.

Chick pineal gland	[^{3}H]Carbo2 (pg/pineal gland)
1	50
2	50
3	30
4	50
5	50

Table II. Amount of [^{3}H]carbo2 collected from five chick pineal glands the middle of the dark phase of an alternate light–dark (12 h: 12 h) program.

Compound	Dose (μM/100 g body weight)	FAT (min)	ST (min)
Placebo	(20 batches)	NA	0
Melatonin	0.5 (2 batches)	NA	0
	1 (5 batches)	NA	0
	2 (5 batches)	NA	0
Pentobarbital	0.5 (3 batches)	NA	0
	1 (2 batches)	13	36
Diazepam	1 (10 batches)	2 to 7	24 to 70
Carbo2	1 (8 batches)	2 to 9	36 to 65
	2 (10 batches)	4 to 11	40 to 70
10-Methoxyharmalan	1.4 (2 batches)	NA	0
Harmaline	1.4 (2 batches)	NA	0

Table III. Hypnotic effects of carbo2, melatonin, and reference compounds. Intramuscular (pectoralis major muscle) administration at 2 PM to chicks under a 7-day alternate light–dark program (LD) (light 8.00 AM to 8.00 PM; dark 8.00 PM to 8.00 AM). NA, not applicable, the animals remained conscious throughout the period of observation; FAT, time taken to fall asleep, equal to the time required to pass from the state of active consciousness to a nonconscious state; ST, sleep time, equal to the duration of the period of sleep from falling asleep to waking up.

Conclusion

We have evidenced the role played by melatonin in both inducing and maintaining nocturnal sleep. Melatonin is the bioprecursor of hypnotic acetyl metabolites, such as carbo2, which result from the enzymatic acetylation of melatonin (and 2-oxomelatonin) by NAT.

Since insomnia and sleep disorders may be due to a lack of NAT enzymes in the pineal gland, a therapeutic approach to sleep disorders could be suggested. Patients with insomnia may be treated by administering hypnotic acetyl metabolites of melatonin or their synthetic analogs. ❑

	Dose (mg/kg)	Duration of observation (min)	Duration (min) (% total sleep)					Latencies (min) (SD)		
			Wake	Drowsiness S_1	SWS $S_2 + S_3$	REM	Total sleep	S_1	SWS	REM
Placebo	0	120	92.6	10.3 (37.6)	14.2 (51.8)	3.5 (12.7)	27.4	66.5 (16.6)	77.0 (9.3)	98.7 (10.3)
Zolpidem	0.62	150	132.1	10.4 (58.1)	7.3 (40.8)	0.2 (1.1)	17.9	106.5 (24.2)	129.3 (22.5)	146.4 (8.8)
Carbo2	1.28	90	32.2	23.6 (40.8)	30.2 (52.2)	4.0 (6.9)	57.8	15.9 (14.8)	27.8 (11.7)	58.1 (14.9)
Carbo2	0.32	90	48.1	17.5 (41.8)	20.2 (48.2)	4.2 (10.0)	41.9	24.5 (9.5)	39.8 (18.6)	75.7 (17.8)
Diazepam	0.20	90	58.5	20.1 (63.8)	10.3 (32.7)	1.1 (3.5)	31.5 (5.0)	24.0	45.5 (23.0)	80.3 (15.6)

Table IV. Polysomnographic recordings of latencies and times spent at each stage of the sleep/wakefulness cycles after intravenous administration of placebo, zolpidem, carbo2, and diazepam to 8 beagles for 90 to 150 min (mean values in 8 dogs). SWS, slow-wave sleep; REM, rapid eye movement; S_1, S_2, S_3, sleep states.

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Papel de la melatonina en la inducción y mantención del sueño

Los estudios farmacocinéticos de la melatonina en voluntarios humanos jóvenes y viejos, y la medición de los efectos hipnóticos en pollos en condiciones de alternancia luz-oscuridad o luz permanente, muestran que la melatonina es un bioprecursor de metabolitos acetilados hipnóticos, los que son producidos por la acetilación enzimática de la melatonina y de la 2-oxomelatonina, ambas bajo control de las N-acetiltransferasas (NATs) que se encuentran en la glándula pineal. El metabolito acetilado de la melatonina, que se denomina carbo2, es una N-acetil-β-carbolina. La arquitectura electroencefalográfica (EEG) del sueño producida por este compuesto es similar al sueño fisiológico y se caracteriza por un porcentaje significativo de sueño profundo de ondas lentas y sueño de movimientos oculares rápidos. Esto está en claro contraste con la arquitectura del sueño EEG observado con compuestos gabaérgicos (GABA, ácido γ-aminobutírico). Ya que se cree que el insomnio y los trastornos del sueño se deben a una falta de enzimas NAT en la glándula pineal, se puede considerar una nueva aproximación terapéutica a los trastornos del sueño al administrar metabolitos acetilados hipnóticos de melatonina o análogos sintéticos de éstos.

Rôle de la mélatonine dans le déclenchement et le maintien du sommeil

Les études pharmacocinétiques sur la mélatonine chez des volontaires humains jeunes et âgés, et la mesure des effets hypnotiques chez des poussins mis dans des conditions alternant lumière/obscurité ou lumière permanente, montrent que la mélatonine est un bioprécurseur de métabolites hypnotiques acétylés produits par l'acétylation enzymatique à la fois de la mélatonine et de la 2-oxo mélatonine sous le contrôle des sérotonine N-acétyltransférases (NAT), qui sont présentes dans la glande pinéale. Le métabolite acétylé de la mélatonine, désigné sous le terme de carbo2, est une N-acétyl-β-carboline. Contrastant nettement avec l'architecture électroencéphalographique (EEG) du sommeil observée avec les composés GABAergiques (GABA, acide γ-aminobutyrique), l'architecture EEG du sommeil produite par le carbo2 est semblable à celle du sommeil physiologique et est caractérisée par la proportion significative de sommeil profond à ondes lentes et de sommeil à mouvements oculaires rapides. Puisque l'insomnie et les troubles du sommeil semblent dus à un déficit pinéal en enzymes N-acétyltransférases, il est possible d'envisager une nouvelle approche thérapeutique des troubles du sommeil par l'administration de métabolites hypnotiques acétylés de la mélatonine ou d'analogues de synthèse.