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Daniel Guillaume, N. Rio, P.L. Toutain. Kinetic studies and production rate of melatonin in pony mares. *AJP - Regulatory, Integrative and Comparative Physiology*, 1995, 268, pp.R1236-R1241. hal-02701727

HAL Id: hal-02701727

<https://hal.inrae.fr/hal-02701727>

Submitted on 1 Jun 2020

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Kinetic studies and production rate of melatonin in pony mares

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Institut National de la Recherche Agronomique de Tours, Physiologie de la Reproduction des Mammifères Domestiques, 37380 Nouzilly; and Ecole Nationale Vétérinaire de Toulouse, Unité Associée Institut National de la Recherche Agronomique de Physiopathologie et Toxicologie Expérimentales, 31076 Toulouse Cédex, France

Guillaume, D., N. Rio, and P. L. Toutain. Kinetic studies and production rate of melatonin in pony mares. *Am. J. Physiol.* 268 (Regulatory Integrative Comp. Physiol. 37): R1236–R1241, 1995.—The aims of the present study were to determine basic kinetic parameters and the nycthemeral production rate of melatonin in the horse. Seven pony mares were used for the kinetic studies. Five other pony mares were used under long and short days for the production rate studies. Melatonin was administered by intravenous, oral, and intragastric routes at different dose levels. The plasma melatonin clearance was $1.02 \pm 0.31 \text{ l} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, and the volume of distribution was $0.89 \pm 0.53 \text{ l/kg}$ for the $0.4 \text{ } \mu\text{g/kg}$ melatonin dose. The systemic availability after oral and intragastric administration was consistently low ($\leq 10\%$). The hourly melatonin production rates during short ($36 \pm 33.6 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and long nights ($42 \pm 24.0 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) were not significantly different. It is concluded that the oral dose of exogenous melatonin required to control reproductive pattern in horses would need to be 10 times higher than the nocturnal melatonin production ($0.5 \text{ } \mu\text{g/kg}$) to obtain a physiological plasma melatonin concentration.

horse; clearance; systemic availability; nocturnal production rate

MELATONIN IS INVOLVED in the determination of the photoperiod and participates in the control of the animal's reproductive cycle both in short-day breeders, such as the ewe (17), and in long-day breeders, such as the mare (8, 11, 22, 23). A photoperiodic treatment with a 14.5:9.5-h light-dark cycle was shown to advance the first ovulation in mares following the winter inactivity (18).

It has also been demonstrated in different species, including the horse, that an administration of exogenous melatonin can be used directly to modulate a photoperiodic treatment and that a 12 mg melatonin dose, 4 h before a stimulating short night, delays the first ovulation in the mare (9).

The rational pharmacological use of melatonin to control reproductive cycles is based on the selection of an appropriate schedule for the time of melatonin administration and an appropriate dosage regimen (dose, interval of administration, and route of administration); the former aspect has been partially documented (10).

The present experiment was designed to determine the basic pharmacokinetic parameters of melatonin, its oral bioavailability, and an effective melatonin dosage regimen in the horse. The physiological melatonin production rate was also determined to assess the physiological meaning of the melatonin-tested doses.

MATERIALS AND METHODS

Animals. Seven Welsh pony mares ($298 \pm 49 \text{ kg}$), 14.4 ± 3 yr old, from the experimental herd at Institut National de la Recherche Agronomique, Nouzilly, were used in the first experiment (melatonin pharmacokinetics). Five pony mares ($239 \pm 60 \text{ kg}$), 7.2 ± 3 yr old, were used in the second experiment (melatonin production-rate determination). Horses were given two equal feedings per day, at 0800 and 1600, that included oats, corn, and lucerne. The morning meal was suppressed, and the afternoon meal was given after the last blood sample on the sampling day.

Photoperiodic treatment. The experiments were carried out at Nouzilly, France, 45°N . The photoperiodic treatment in all mares started at the end of December. Mares in the pharmacokinetic experiment were maintained under long days (14.5:9.5-h light-dark). Those in the production-rate experiment were kept under short days (in lightproof boxes) (10.5:13.5-h light-dark) until the first blood sampling session at the end of April. They were then maintained under long days (14.5:9.5-h light-dark), the second blood sampling session being carried out 1 mo later.

Melatonin administration and blood sampling. Crystalline melatonin (Sigma Chemical, St. Louis, MO) was used. Purity ($>98\%$) was checked by thin-layer chromatography. Melatonin was administered by intravenous, oral, and intragastric routes. The order of administration was randomized according to a Latin square with seven periods. An interval of at least 1 wk was allowed to elapse between each period.

For the two intravenous administrations, melatonin was dissolved in an appropriate volume of absolute ethanol (Pro-labo, Normapur, Paris, France), so that a volume of $\sim 5 \text{ ml}$ could be administered.

For the oral administration trials, an appropriate volume of alcoholic solution, based on individual mare body weight, was spread on a sugar lump ($\sim 7 \text{ g}$). The sugar lumps were left at room temperature to evaporate the ethanol, then stored at -20°C until administration. The stability of melatonin at this temperature was demonstrated (9).

The melatonin solution was administered in the left jugular vein with a sterilized Teflon trocar (Intraflon, Vigon, Paris, France). Two doses (40 and $0.40 \text{ } \mu\text{g/kg}$ iv) were administered in the pharmacokinetic experiment. Blood samples were collected from the right jugular vein in heparinized tubes (sodium heparinate) with a sterilized Teflon trocar. Blood samples were obtained before (-20 min , -10 , and 0 min) and after (1 , 2 , 4 , 6 , 8 , 10 , 12 , 16 , 20 , 25 , 30 , 35 , 40 , 45 , 60 , 75 , 90 , 120 , 150 , 180 , 210 , 240 , 300 , 360 , 420 , 480 , 540 , and 660 min) intravenous melatonin administration.

Melatonin was administered orally on a piece of sugar, as the horse had been trained to accept sugar spontaneously. The four dose levels (40 , 4.0 , and 0.4 or $0.08 \text{ } \mu\text{g/kg}$) were administered to each mare in the morning at 8 A.M., after switching on the light. For the intragastric administration trial, a $40 \text{ } \mu\text{g/kg}$ dose in alcoholic solution (5 ml) was administered to each mare with a Teflon catheter of appropriate length introduced into

the nasostomach tube. A twitch was used to keep the horse under control during administration. Blood samples were obtained as with intravenous administration and according to the same schedule.

For the production-rate experiment, two series of jugular blood samples (corresponding to short and long days, respectively) were collected over 24 h. Blood samples were obtained every 6 min, 18 min before switching off the light until 18 min after completion of the dark phase and hourly during the rest of the session. A cover was placed over the eyes of the mares, and a dim red light was used during the dark-phase blood collection.

For this second experiment, a single dose (0.8 µg/kg iv) was administered 2 mo after the second blood-sampling session to determine the individual plasma melatonin clearance in pony mares. Blood samples were taken as in the pharmacokinetic experiment. Blood was immediately centrifuged, and the plasma was frozen at -20°C until assayed.

Melatonin assays. The melatonin plasma concentration was measured by adapting a specific radioimmunoassay technique previously described in sheep. The cross-reaction with related compounds was <1% for *N*-acetylserotonin and 6-hydroxymelatonin and totally absent for other compounds tested (24).

The assay was direct with ¹²⁵I-labeled melatonin. Precipitation of the bound fraction was obtained with ovine serum raised against rabbit γ-globulin. It was adapted to the horse plasma by preparing a standard curve (5–2,000 pg/ml) in low-melatonin equine plasma. The melatonin in this low-melatonin plasma was as low as the plasma in which the melatonin had been removed by charcoal treatment. The concentrations were calculated by two logit (B/B₀) vs. log (dose) regressions; the first from 5 to 500 pg/ml and the second from 500 to 2,000 pg/ml. All plasma samples from each horse were assayed at the same time. The pharmacokinetic and production rate trials were determined in two different series. The level of quantification was 5 pg/ml, and the intra-assay coefficients of variation were <10% at the level of quantification (5 pg/ml) and <5% for physiological melatonin concentration (40–60 pg/ml). The interassay coefficient of variation was <13%.

Pharmacokinetic analysis. The pharmacokinetics of the melatonin plasma concentrations were analyzed using a program for nonlinear regression analysis adapted from MULTI analysis program (28).

Plasma melatonin concentrations obtained after intravenous melatonin administration were fitted to the general polyexponential equation (Eq. 1)

$$C(t) = \sum_{i=1}^n Y_i \exp(-\lambda_i t) \quad (1)$$

In Eq. 1, $C(t)$ is the melatonin plasma concentration (pg/ml) at time t (min), Y_i (pg/ml) is the coefficient of the exponential term, and λ_i (min⁻¹) is the exponential term; initial estimates were obtained using the residuals method (7). The data points were weighted by the inverse of the squared-fitted value ($1/Y_i^2$).

The best fit was obtained by minimizing the weighted least-squares criteria, and the number of exponents (1, 2, or 3) needed for each data set was determined by application of the Akaike's information criterion (26). Based on this criterion, a triexponential equation was selected for the intravenous administration (Eq. 2)

$$C(t) = Y_1 \exp(-\lambda_1 t) + Y_2 \exp(-\lambda_2 t) + Y_3 \exp(-\lambda_3 t) \quad (2)$$

In Eq. 2, $C(t)$ is the melatonin concentration at time t ; Y_1 , Y_2 , and Y_3 (pg/ml) are coefficients; and λ_1 , λ_2 , and λ_3 (min⁻¹) are

exponents. Data were therefore analyzed using a three-compartment open model with melatonin elimination from the central compartment.

The estimated parameters (Y_1 , Y_2 , Y_3 , and λ_1 , λ_2 , λ_3) were used to solve the first-order rate constants of transfer from central to peripheral compartments (K_{12} , K_{13} , K_{21} , K_{31}) using classical equations (7).

The volume of the central compartment was obtained with Eq. 3

$$V_c = \text{dose}/(Y_1 + Y_2 + Y_3) \quad (3)$$

with dose, the administered dose (0.4 or 40 µg/kg), and Y_1 , Y_2 , and Y_3 , as previously defined.

The melatonin steady-state volume of distribution (V_{ss}) was obtained using Eq. 4

$$V_{ss} = V_c (1 + K_{12}/K_{21} + K_{13}/K_{31}) \quad (4)$$

with V_c , the volume of the central compartment, as given in Eq. 3, and K_{12} , K_{13} , K_{21} , and K_{31} , the first-order rate constants transfer between central and peripheral compartments.

The plasma melatonin clearance (CL) was calculated using Eq. 5

$$CL = \text{dose}/AUC \quad (5)$$

In Eq. 5, AUC is the area under the plasma melatonin curve obtained by the trapezoidal method without extrapolation to infinity.

The terminal half-life ($t_{1/2}$) was obtained using Eq. 6

$$t_{1/2} = \ln(2)/\lambda_3 \quad (6)$$

with λ_3 as the slope of the terminal phase.

The mean residence time (MRT) of melatonin in the system, i.e., the mean time taken for a melatonin molecule to transit through the body, was calculated by the trapezoidal method without extrapolation to infinity (27).

Individual plasma melatonin concentrations obtained after oral and intragastric melatonin administrations were best fitted (Akaike's information criterion) using Eq. 7

$$C(t) = (Y_1 + Y_2) \exp(-K_a t) + Y_1 \exp(-\lambda_1 t) + Y_2 \exp(-\lambda_2 t) \quad (7)$$

In Eq. 7, $C(t)$ is the plasma melatonin concentration (pg/ml) at time t (in min); Y_1 and Y_2 (pg/ml), the pre-exponential coefficients; λ_1 and λ_2 (min⁻¹), the exponents corresponding to the distribution and elimination processes; and K_a (min⁻¹), the first-order rate constant of absorption. The data were therefore described by a two-compartment model with a single process of absorption.

The plasma half-life ($t_{1/2}$) was calculated using Eq. 8

$$t_{1/2} = \ln(2)/\lambda_2 \quad (8)$$

with λ_2 the slope of the terminal phase.

T_{max} , the time corresponding to the occurrence of the maximal plasma concentration (C_{max}), was calculated from Eq. 7. The systemic availabilities (F%) of melatonin after the oral and intragastric administrations were calculated using Eq. 9

$$F\% = \frac{AUC_{(PO \text{ or } IG)} \times \text{dose}_{(IV)}}{AUC_{(IV)} \times \text{dose}_{(PO \text{ or } IG)}} \times 100 \quad (9)$$

with AUC, the area under the curve after oral (PO), intragastric (IG), and intravenous (IV) administration. The 0.40 µg/kg IV dose was used to calculate the absolute bioavailability.

The mean absorption times (MAT) after PO and IG administration were calculated using Eq. 10

$$\text{MAT}_{(\text{PO or IG})} = \text{MRT}_{(\text{PO or IG})} - \text{MRT}_{(\text{IV})} \quad (10)$$

with $\text{MRT}_{(\text{IV})}$, the MRT calculated after the 0.40 $\mu\text{g/kg}$ iv dose, and $\text{MRT}_{(\text{PO or IG})}$, the MRT calculated after oral or intragastric administration, respectively.

Melatonin production rate. The daily production rate (PR) of melatonin was calculated using Eq. 11

$$\text{PR} = \text{AUC}_{(0-t)} \times \text{CL} \quad (11)$$

In Eq. 11, $\text{AUC}_{(0-t)}$ is the area under the plasma melatonin curve for the night duration (9.5 or 13.5 h) and was calculated by linear trapezoidal rule; CL is the individual plasma clearance calculated after a dose of 0.80 $\mu\text{g/kg}$ iv of melatonin (see Eq. 5).

Statistical analysis. Statistical analysis was performed using STATGRAPHICS (STSC, Rockville, MD). Values are reported as means $\pm$ SD. For the intravenous studies, parameter comparisons between doses were based on an analysis of variance (ANOVA) with doses and horses as the main factors, followed by the Bonferroni test for comparison between the means. Homogeneity of variance was tested using the Bartlett's test. Nonparametric analysis of variances (Friedman two-way analysis) was carried out when the variances were not homogeneous.

For the oral studies, ANOVA, with doses and horses as main factors, were carried out using the same procedure as for the intravenous studies. Differences were considered as significant for $P < 0.05$.

RESULTS

Plasma melatonin kinetics. Results are only available for six horses, because of difficulties encountered with one of the horses. The semilogarithmic plots of melatonin plasma concentrations (pg/ml) vs. time (in min) after the intravenous administration (40 and 0.40 $\mu\text{g/kg}$) are shown in Fig. 1 for a representative horse.

The plasma clearance calculated after the 40 $\mu\text{g/kg}$ melatonin dose ($1.83 \pm 0.39 \text{ l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) was significantly higher than after the 0.40 $\mu\text{g/kg}$ dose ($1.02 \pm 0.31 \text{ l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) (Bonferroni test, $P < 0.01$). The mean steady-state volume of distribution was higher ($2.11 \pm 1.75 \text{ l/kg}$) for the 40 $\mu\text{g/kg}$ dose than for the 0.40 $\mu\text{g/kg}$ dose ($0.89 \pm 0.53 \text{ l/kg}$), but the difference was not statistically significant (Friedman analysis,

Fig. 1. Semilogarithmic plot of observed plasma melatonin concentration after an intravenous administration of melatonin at a dose of 40 (■) or 0.4 $\mu\text{g/kg}$ (□) to a representative horse.

$P > 0.05$); finally, MRTs, which depend on both clearance and volume of distribution, did not differ significantly between the two doses (68 ± 53 and 58 ± 46 min for the high- and low-melatonin doses, respectively; ANOVA, $P > 0.05$). Other parameters are given in Table 1.

The semilogarithmic plots of melatonin plasma concentrations vs. time (in min) for the oral administrations (40, 4.0, and 0.4 $\mu\text{g/kg}$) are shown in Fig. 2 for a representative horse. The plasma melatonin concentration after oral administration of 0.08 $\mu\text{g/kg}$ was undetectable. The plasma melatonin concentration increased rapidly after administration of the three tested oral doses, to reach a maximum value (T_{max}) after ~ 30 min. There was no statistical difference between doses for T_{max} (ANOVA, $P > 0.05$); in contrast, the maximum plasma melatonin concentration was proportional to the administered dose, suggesting linearity of melatonin absorption (Table 2). The terminal half-lives (~ 100 – 110 min) were similar for the three dosage levels and those of the intravenous studies (108–138 min) (ANOVA, $P > 0.05$), suggesting that melatonin absorption occurred more rapidly than melatonin elimination. The MAT, i.e., the mean time taken for a melatonin molecule to be absorbed, was 50 ± 15 (40 $\mu\text{g/kg}$ dose) and 33 ± 17 min (4.0 $\mu\text{g/kg}$ dose). The MAT for the lowest dose could only be calculated in two horses and is not reported. The mean systemic availability (for all oral dose levels) was low (~ 8 – 12%) with no significant difference between dosage rates (ANOVA, $P > 0.05$). Mean parameters are given in Table 2 for the three oral doses tested. The plasma melatonin concentration was immediately increased after an intragastric melatonin administration (40 $\mu\text{g/kg}$), and maximal plasma values were observed at the first sampling time (1-min postadministration) in four of the six horses (see DISCUSSION). The bioavailability was significantly lower ($4.1 \pm 2.4\%$) after the intragastric administration than after oral administration of the same dose ($8.8 \pm 3.3\%$) (paired t -test, $P < 0.05$).

Melatonin production rate. The melatonin kinetic parameters for the five horses used to evaluate melatonin production rate are given in Table 1. The plasma clearance ($1.30 \pm 0.28 \text{ l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) was not significantly different from the clearance calculated with the 0.4 $\mu\text{g/kg}$ dose ($1.02 \pm 0.31 \text{ l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). None of the other parameters (V_{ss} , V_c , MRT, and half-life) was significantly different from those calculated for the 0.4 $\mu\text{g/kg}$ dose administered to the six other horses (t -test, $P > 0.05$).

The secretion pattern of a representative mare is given in Fig. 3. Total melatonin production rate was higher during a long night of 13.5 h ($569 \pm 325 \text{ ng/kg}$) than during a short night of 9.5 h ($343 \pm 317 \text{ ng/kg}$) (paired t -test, $P < 0.1$), but the hourly melatonin production rates (36 ± 33.6 and $42 \pm 24.0 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) did not differ significantly between the two types of night (paired t -test, $P > 0.05$).

DISCUSSION

The rational use of melatonin to control reproductive patterns in mares requires, for a given route of administration, determination of the appropriate dosage regi-

Table 1. Pharmacokinetic parameters of melatonin after an intravenous bolus administration of melatonin at different dose levels in two groups of pony mares

Parameters	Pharmacokinetic Trial, $\mu\text{g/kg}$ ($n = 6$)		Statistical Significance of Dose	Production- Rate Trial, $0.8 \mu\text{g/kg}$ ($n = 5$)
	40	0.4		
Clearance, $\text{l} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$	1.83 ± 0.39	1.02 ± 0.31	$P < 0.01$	1.30 ± 0.28
V_c , l/kg	1.04 ± 0.80	0.35 ± 0.15	NS	0.43 ± 0.10
V_{ss} , l/kg	2.11 ± 1.75	0.89 ± 0.53	NS	1.92 ± 0.23
Half-life, in min	138 (99–178)	108 (51–170)	NS	109 (47–282)
MRT, in min	68 ± 53	58 ± 46	NS	42 ± 3.8
AUC, $\mu\text{g} \cdot \text{min} \cdot \text{ml}^{-1}$	$1,370 \pm 330$	24.9 ± 0.61	NC	38.4 ± 0.97

Values are means $\pm$ SD; values in parentheses are ranges. V_c , initial volume of distribution; V_{ss} , steady-state volume of distribution; MRT, mean residence time; and AUC, area under curve. Clearance, MRT, and AUC were calculated by the trapezoidal rule without extrapolation to infinity; V_c and V_{ss} were calculated using compartmental modeling; NS, not significant; NC, not calculated. Pharmacokinetic data were subjected to statistical analysis using an analysis of variance (ANOVA) with two factors (horse, dose). Comparison by *t*-test showed that there was no significant difference between the $0.4 \mu\text{g/kg}$ dose (pharmacokinetic trial) and the $0.8 \mu\text{g/kg}$ dose (production-rate trial).

men. In the horse, the oral route is most convenient when long-term treatment is in order, especially if mimicking the natural endogenous rhythm is a prerequisite to melatonin efficacy, as has been shown in the gilt to overcome delayed puberty (20). Because of the particularity of the digestive physiology in horses, however, oral-route efficiency, in terms of systemic bioavailability, may vary considerably depending on the drug tested. Pharmacokinetics are generally used to establish the optimal dosage regimen when a therapeutic drug level has been determined. Effective plasma melatonin levels can be assumed, as a result of experiments with photoperiod, to be equal to the physiological melatonin levels observed during the night. In other words, the systemically available fraction of melatonin following an exogenous administration must be of the same order of magnitude as the spontaneous melatonin production rate. Our experiment was therefore designed to document both oral-route performance and the physiological production rate of melatonin in horses.

Clearance is a basic pharmacokinetic parameter, which expresses the body's capacity to eliminate and/or metabolize a drug. In the present experiment, the estimated plasma clearance in five horses (production-rate studies) was $1.31 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, which is very similar to that found

in cows ($1.44 \text{ l} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) (2). This value is also similar to the hepatic plasma flow in horses (4). As the main pathway for melatonin metabolism in humans and rodents is hepatic (12, 13), the calculated clearance value in the horse suggests the existence of a first-pass hepatic effect for melatonin, as reported by others (19). One consequence of the first-pass hepatic effect is that the oral bioavailability of melatonin should be $< 100\%$ (see below).

Kinetic parameters were obtained during the day in the present experiment. Mid-dark melatonin clearance was reported to be higher than midnight clearance in rats (15). However, as a circadian rhythm of melatonin clearance has not been reported in humans (15), cows (3), or sheep, (5) we assumed that the calculated clearance obtained during the light phase was identical to that obtained during the night for the production-rate calculation.

Melatonin clearance was shown to be dose dependent in the six other horses, the clearance obtained with the highest doses being higher than that obtained with the lowest dose (1.83 compared with $1.02 \text{ l} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for the 40 and $0.4 \mu\text{g/kg}$ doses, respectively). Similarly, the steady-state volume of distribution was higher (2.11 vs. 0.89 l/kg) with the highest melatonin dose. These results were rather unexpected, as melatonin kinetics were reported to be linear (dose independent) in other species, including sheep (5), humans (15), and rats (6). The clearance values obtained in the present experiment with the lowest tested doses ($0.40 \mu\text{g/kg}$ with 6 horses and $0.8 \mu\text{g/kg}$ with 5 other horses) were not significantly different and can be considered as physiological, as they were obtained with melatonin doses of the same order of magnitude as the physiological production rate of melatonin (see below). In contrast, the $40 \mu\text{g/kg}$ melatonin dose gave much higher than physiological plasma concentrations of melatonin and led to some nonlinearity in melatonin disposition (increasing the volume of distribution, metabolism, and/or elimination). This point must be taken into account when production rates or absolute bioavailability of melatonin are measured in horses, and we suggest that the reference intravenous administration should be $< 1 \mu\text{g/kg}$. It should be noted that the plasma half-life (108 – 138 min) was not affected by the tested doses. This is because plasma half-life is a hybrid

Fig. 2. Semilogarithmic plot of plasma melatonin concentration after an oral administration of melatonin at a dose of 40 (■), 4 (□) or $0.4 \mu\text{g/kg}$ (●).

Table 2. Pharmacokinetic parameters of melatonin after oral administration of melatonin at three different dose levels and a nasogastric administration in six pony mares

Parameters	Oral Administration, $\mu\text{g/kg}$			Statistical Significance of Dose	Intragastric Administration, 40 $\mu\text{g/kg}$
	40	4	0.4		
Half-life, in min	105 (68–149)	101 (51–199)	108 (30–309)	NS	NC
MRT, in min	89 $\pm$ 16	71 $\pm$ 13	53 $\pm$ 23	$P < 0.01$	89 $\pm$ 20
MAT, in min	50 $\pm$ 15	33 $\pm$ 17	NC	NC	47 $\pm$ 23
C_{max} , pg/ml	2,886 $\pm$ 1,706	283 $\pm$ 118	47 $\pm$ 23	NC	1,137 $\pm$ 710
T_{max} , in min	31 $\pm$ 7	32 $\pm$ 11	23 $\pm$ 13	NS	30 $\pm$ 1.73
AUC, $\mu\text{g} \cdot \text{min} \cdot \text{ml}^{-1}$	282 $\pm$ 138	26 $\pm$ 12	3.71 $\pm$ 3.12	NC	119 $\pm$ 78
F%	8.8 $\pm$ 3.3	9.8 $\pm$ 4.8	12.0 $\pm$ 4.6	NS	4.1 $\pm$ 2.4

Values are means $\pm$ SD; values in parentheses are ranges. MAT, mean absorption time; C_{max} , maximal plasma concentration; T_{max} , time of peak plasma concentration; and F%, systemic availability in percent. MRT, MAT, and AUC were calculated by trapezoidal rule without extrapolation to infinity; NS, not significant; NC, not calculated. Oral route data were subjected to statistical analysis using an ANOVA with two factors (horse, dose). Comparison of oral and intragastric administration of 40 $\mu\text{g/kg}$ by a paired t -test revealed a significant difference between bioavailabilities ($P < 0.05$).

parameter, proportional to the volume of distribution and inversely proportional to clearance (7). It should also be noted that the volume of distribution (V_{ss}) was relatively large (~ 1 l/kg), suggesting an intracellular penetration of melatonin; this is consistent with the lipophilicity of the molecule and its ability to penetrate the cell and act directly on cell physiology, probably via calmodulin (1).

Fig. 3. Plasma profile of melatonin concentration during a short (■) or a long (□) night in a representative horse. The dark phase (9.5 or 13.5 h) is represented by the hatched bars.

The melatonin production rate in horses was ~ 0.6 – 0.7 ng $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$, i.e., a total production of 343–569 ng/kg for night durations of 9.5 and 13.5 h, respectively. These values are similar to those reported in cows (2) and humans (13), and pharmacological control of the reproductive pattern using melatonin in mares can be expected to require a systemic available melatonin dose of this order of magnitude. The instantaneous melatonin production rates during short and a long nights (9.5 and 13.5 h, respectively) were also similar, so the total production rate was directly proportional to the night duration; this is at variance with observations in the Syrian hamster, in which the melatonin production rate increased sharply at the end of the night (21).

Calculation of a suitable dosage regimen of melatonin for use in horse should therefore be based only on the required pharmacological night duration and an appropriate route of administration able to provide a constant melatonin delivery.

The present experiment clearly demonstrates that, when melatonin is administered orally, the dosage level bioavailability factor must be adjusted to the bioavailability. The absolute bioavailability for all three tested doses (40, 4.0, and 0.4 $\mu\text{g/kg}$) was $< 10\%$. Such low availability is probably a consequence of both a first-pass hepatic effect and incomplete absorption as in humans, in whom the systemic availability was also reported to be $< 10\%$ (13). The absolute bioavailability was similar for all three doses, suggesting the linearity of melatonin absorption. A dose of ~ 5 $\mu\text{g/kg}$ would thus be required to provide ~ 0.5 $\mu\text{g/kg}$ of available melatonin, i.e., an amount equivalent to the nocturnal production rate of melatonin in horse. Melatonin absorption after oral administration occurred rapidly in the present experiment. The time of maximal concentration (~ 300 pg/ml for the 4.0 $\mu\text{g/kg}$ dose) was observed after 30 min. In contrast, a delay of ~ 3 h was required after the same dose to return to a mean plasma level of 30 pg/ml, i.e., a concentration equivalent to the calculated mean plasma night concentration. A 4.0 $\mu\text{g/kg}$ melatonin dose by oral route can therefore be assumed to be equivalent to a supplementary night duration of ~ 3 h (with respect to an appropriate time of administration) in horse. In

humans, it was estimated that a melatonin dose from 1.0 to 1.5 $\mu\text{g}/\text{kg}$ was enough to raise blood melatonin to nocturnal concentrations (25). This is in agreement with our result, as the oral bioavailability, plasma clearance, and melatonin production rate in humans (13) are of the same order of magnitude as those of the horse.

A final recommendation concerning the dose level to be administered by oral route cannot be made from the present experiment, as bioavailability depends on many factors, including the formulation and horse digestive status. It is not certain that melatonin directly incorporated into food will necessarily be 10% bioavailable, as with the sugar lump used in the present experiment. The absolute bioavailability after an intragastric administration, which more closely resembles the state of melatonin administration incorporated into food, was only 4%. This suggests that oral administration on a sugar lump results in some perlingual absorption of melatonin, thus avoiding a hepatic first-pass effect. The condition of melatonin administration with respect to food intake itself is probably important too. It has been shown that drug administration before a meal leads to rapid absorption, whereas administration at the end of the meal delays absorption for several hours (14). This is because gastric emptying is only apparent during food intake in horse, and gastric emptying is a prerequisite for the absorption of drugs from the gut.

In conclusion, the present experiment demonstrates that the systemic availability of melatonin after oral administration is very low in the horse and that a pharmacological control of reproductive pattern, based on oral melatonin administration, must be carried out using a dosage regimen at least 10 times higher than the physiological melatonin production rate in this species. A supplementary 3-h pharmacological night can be expected with such a dosage.

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Received 7 December 1993; accepted in final form 17 November 1994.

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