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Nicorandil affects diurnal rhythms of body temperature, heart rate and locomotor activity in rats

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Abstract

The effects of nicorandil, a K^+ channel opener with a potent vasodilator action, on diurnal rhythms of body temperature, heart rate and locomotor activity were assessed in rats. Transmitters were intraperitoneally implanted under ether anaesthesia. After recovery from surgery, body temperature, heart rate and locomotor activity were recorded during control, saline or nicorandil (10 $mg \cdot kg^{-1}$ administered orally) treatment and for 5 days after treatment. For each period, Fourier analysis determined the predominant rhythmicity for body temperature, heart rate and locomotor activity while cosinor analysis assessed the corresponding mesors, acrophases and amplitudes and maxima and minima were directly plotted from raw data. The results indicated: (1) loss of the diurnal rhythmicity for all three rhythms after implantation; (2) stress-induced modifications of almost all the characteristics of the three rhythms after saline and (3) a loss of diurnal rhythmicity of heart rate after nicorandil, an effect that was not observed after saline and which was reversed when nicorandil administration was stopped. In conclusion, nicorandil perturbed the diurnal rhythmicity of heart rate while the rhythmicity of body temperature and locomotor activity was not affected. © 1998 Elsevier Science B.V.

Keywords: Nicorandil; Telemetry; Diurnal rhythm; Body temperature; Heart rate; Locomotor activity; (Rat)

1. Introduction

Nicorandil (2-nicotinamidoethyl nitrate), one of the most commonly used K^+ channel agonists, has a potent vasodilator action and acts preferably on coronary artery smooth muscles (Longman et al., 1988; Hiraoka and Fan, 1989). Opening of K^+ channels results in hyperpolarization of the membrane potential, inhibition of Ca^{2+} influx and vasodilatation. Besides its K^+ channel opening property, nicorandil also possesses a guanylate cyclase activity which leads to relaxation (Edwards and Weston, 1990; Quast, 1992). The most striking action of nicorandil on cardiac muscle is a shortening of the action potential duration (Hamilton and Weston, 1989). Nicorandil has been reported as being responsible for an increase in heart rate (Duty and Weston, 1990) and is therefore likely to influence the diurnal rhythmicity of the heart rate. It is well known that temperature, heart rate and locomotor activity, like many other physiological and behavioral measures, fluctuate with a daily, or circadian rhythm, (Van den Buuse, 1994). As the interrelationship among heart rate, activity and body temperature in the rat has been widely described by Meinrath and D'Amato (1979), it was of interest to evaluate the influence of repeated nicorandil administration on diurnal rhythms of body temperature, heart rate and locomotor activity in unrestrained rats by using radiotelemetry with implantable transmitters.

2. Materials and methods

2.1. Animals

For a minimum of three weeks before use, 5 Wistar AF IOPS male rats (mean weight = 267 g) obtained from IFFA-CREDO (St. Germain-sur-L'Arbresle, France) were individually caged in the same room under controlled conditions: humidity (50–55%), temperature 24 ± 1°C and a 12 h light–dark cycle (L:D 12:12; L from 06.00 to 18.00) with free access to food (UAR) and water.

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2.2. Drug

Nicorandil (2-nicotinamidoethyl nitrate) was obtained from Merck Laboratories and was dissolved in saline.

2.3. Protocol of the study

The study, which was conducted during the month of June, was divided into four periods of 5 days each: (1) day 6–10: baseline measurements of body temperature, heart rate and locomotor activity, (2) day 11–15: daily oral administration of saline, (3) day 16–20: daily oral administration of 10 mg/kg of nicorandil and (4) day 21–25: post treatment period. Saline as well as nicorandil were given orally, via a tube into the stomach, in a volume of about 0.8 ml at 08.00 h. The experimental study was conducted under light–dark conditions.

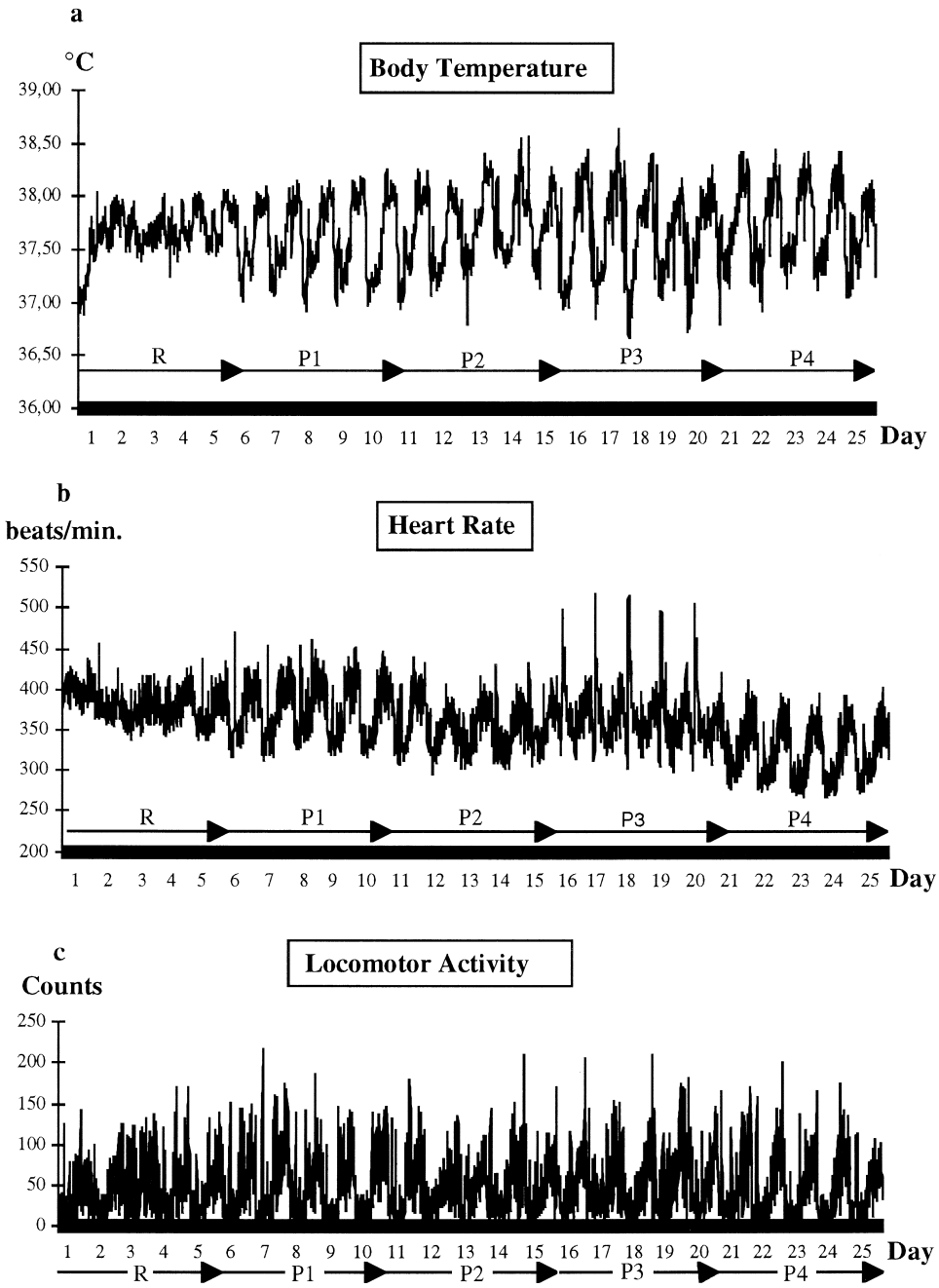

Fig. 1. Time-courses of body temperature (a), heart rate (b) and gross locomotor activity (c) over the 25-day period including recovery from surgery (R), baseline measurements (P1), saline (P2), nicorandil (P3) and post-treatment (P4) periods. Data were plotted every 10 min and expressed as means for 5 rats. The experimental study was conducted under light–dark cycle (L:D 12:12; L from 06.00 to 18.00) which is, for convenience, not represented here.

Table 1
Fourier analysis

	Recovery			Baseline			Saline			Nicorandil			Post-treatment		
	H	T	A	H	T	A	H	T	A	H	T	A	H	T	A
Rat 1	170.43	21.21	170.43	24.24	24.24	21.21	21.21	21.21	8.33	8.09	21.21	24.24	24.24	21.21	24.24
Rat 2	56.53	42.40	24.24	24.24	24.24	21.21	24.24	24.24	24.24	5.53	21.21	24.24	—	—	—
Rat 3	170.43	42.40	21.38	24.24	24.24	24.24	24.24	24.24	24.24	21.21	24.24	24.24	21.21	21.21	24.24
Rat 4	21.21	42.40	56.53	24.24	24.24	24.24	24.24	24.24	24.24	24.24	24.24	21.21	21.21	24.24	21.21
Rat 5	56.53	170.43	24.24	24.24	24.24	24.24	24.24	24.24	24.24	170.43	24.24	24.24	21.21	21.21	24.24

Fourier analysis was performed for heart rate (H), body temperature (T) and locomotor activity (A) for each rat and each period and the dominant rhythm was expressed in h min.
— indicates that Fourier analysis could not be performed for this rat.

2.4. Telemetry system

Body temperature, heart rate and locomotor activity were measured by means of radiotelemetry. In accordance with the European recommendations governing the protection of animals used for experimental purposes, the transmitters (model TA11-CTA-F40®, Data Sciences, St. Paul, MN) were implanted intraperitoneally under ether general anaesthesia as described in detail by Kramer et al. (1993) and Gordon (1994b). Briefly, after full anaesthesia was achieved, an incision was made in the peritoneum and the telemetry sender was implanted in the abdominal cavity and sutured to the abdominal wall. Electrocardiogram leads were extended subcutaneously to the right axilla and to the left lower rib area and sutured to muscle tissue there. After closure, the animals were monitored until recovery from anaesthesia and were then returned to their home cages. Signals from the transmitter were received by an antenna mounted in a receiver board (model CTR86®, Data Sciences) placed under the animal's cage. Data were multiplexed at a consolidation matrix (model BCM-100®, Data Sciences) with data from 5 animals. Data for body temperature (°C), heart rate (beats/min) and locomotor activity (counts) were collected, as described by Meerlo et al. (1996), every 10 min over a 25-day period and processed by a PC with a specialized recording and analysis system (Dataquest III®, Data Sciences). The animals were allowed to recover from the surgical stress from day 1 to 5.

2.5. Data analysis

The *n*-values listed in the text represent the number of animals for which the study was performed. The 5-day time series for each rat were analysed by two methods, using Dataquest III® software. Firstly, in order to determine the dominant period of rhythmicity, power spectrum analysis (Fourier transform) was applied to 20 min average data intervals. The method of cosinor analysis (least-square cosine regression) was used to determine phase markers of biological rhythms (Refinetti, 1992) and was therefore applied to the different periods in order to assess mean ± S.E.M. diurnal variables. This method involves the fitting of a cosine wave to the raw data, after which the diurnal rhythm characteristics of body temperature, heart rate and

Table 2
Cosinor analysis: mesors, amplitudes, maxima and minima of heart rate (*H*), body temperature (*T*) and locomotor activity (*A*)

		Mesor ± S.E.M.	Amplitude ± S.E.M.	Maxima ± S.E.M.	Minima ± S.E.M.
P1	*H* (b/m)	$375 \pm 2^{a,b}$	37 ± 2^{a}	$487 \pm 4^{a,b}$	285 ± 4^{a}
	T (°C)	37.62 ± 0.03	0.41 ± 0.02	38.38 ± 0.06	36.77 ± 0.04
	A (counts)	51.37 ± 2.55	35.13 ± 1.83	303.88 ± 17.85	0.00 ± 0.00
P2	*H* (b/m)	355 ± 3	28 ± 2	464 ± 5^{b}	274 ± 4^{b}
	T (°C)	37.72 ± 0.03	0.41 ± 0.02	38.64 ± 0.07^{c}	36.65 ± 0.12
	A (counts)	45.98 ± 2.62	26.22 ± 1.68^{c}	271.64 ± 13.55	0.00 ± 0.00
P3	*H* (b/m)	$367 \pm 4^{a,b}$	$24 \pm 2^{c,b}$	$511 \pm 5^{a,b,c}$	280 ± 4^{b}
	T (°C)	37.67 ± 0.04	0.52 ± 0.04	38.73 ± 0.09^{c}	36.44 ± 0.11
	A (counts)	50.76 ± 4.37	29.04 ± 2.36^{c}	311.08 ± 19.08	0.00 ± 0.00
P4	*H* (b/m)	322 ± 2^{a}	37 ± 2^{a}	438 ± 6	259 ± 1^{c}
	T (°C)	37.75 ± 0.06	0.43 ± 0.04	38.74 ± 0.12^{c}	36.61 ± 0.15
	A (counts)	43.23 ± 2.04	30.79 ± 2.57	273.50 ± 14.99	0.00 ± 0.00

Results are means ± S.E.M. for 5 rats during baseline measurement (P1), saline (P2) nicorandil (P3) and the post-treatment periods (P4). Statistics by Fisher LSD tests: [a]different from P2 (saline): $P < 0.05$; [b]different from P4 (post-treatment): $P < 0.05$ and [c]different from baseline (P1): $P < 0.05$.

Table 3
Cosinor analysis: acrophases of body temperature, heart rate and locomotor activity

	Body temperature (h min)	Heart rate (h min)	Locomotor activity (h min)
Baseline	23.52 ± 0.08	23.38 ± 0.11	24.48 ± 0.11
Saline	01.06 ± 0.26^{c}	24.26 ± 0.23^{c}	02.09 ± 0.42^{c}
Nicorandil	01.20 ± 0.24^{c}	$07.23 \pm 0.16^{a,b,c}$	$04.12 \pm 0.20^{a,b,c}$
Post-treatment	24.51 ± 0.16^{c}	24.11 ± 0.12	02.04 ± 0.15^{c}

Results are means $\pm$ S.E.M. for 5 rats during baseline measurements, saline, nicorandil and the post-treatment periods. Statistics by Fisher LSD test: [a]different from saline: $P < 0.05$; [b]different from post-treatment: $P < 0.05$ and [c]different from baselines: $P < 0.05$.

locomotor activity, such as mesors (midline estimating statistic of rhythm), acrophases (time at which the fitted curve reaches its peak, given in h and min in terms of clock time) and amplitudes (1/2 of the peak-to-through difference of the fitted cosine), were assessed. Finally, minima and maxima of body temperature, heart rate and locomotor activity were computed from individual raw data. All variables were evaluated by using an analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test for multiple comparisons when ANOVA indicated significant differences between groups. A value of $P < 0.05$ was considered to indicate a significant effect.

3. Results

Fig. 1a, b and c presents the time courses of body temperature, heart rate and locomotor activity respectively over the 25-day period. Data were plotted every 10 min and expressed as means ($n = 5$).

3.1. Fourier analysis

Fourier analysis (Table 1) as well as Fig. 1a, b and c showed a loss of the diurnal rhythms of body temperature, heart rate and locomotor activity after surgery and a recovery of these rhythms during baseline measurements and saline administration. Furthermore, Fourier analysis revealed the loss of diurnal rhythmicity of heart rate during nicorandil treatment in 3 animals and recovery of this rhythm during the post treatment period.

3.2. Cosinor analysis

When Fourier analysis detected a diurnal rhythm, cosinor analysis was performed for the detected periodicity. When no diurnal rhythmicity was detected (as seen for heart rate in the treatment period) cosinor analysis assessed mesors and amplitudes for the cosine function corresponding to the detected ultradian rhythm. Further, only acrophases of rats that showed diurnal rhythmicity were included in the data analysis.

Cosinor analysis (Table 2) revealed a significant decrease in the mesor, amplitude, maxima and minima of heart rate following saline administration when compared to baseline measurements. Furthermore, the maxima and acrophase of body temperature as well as the amplitude and acrophase of locomotor activity were significantly modified by saline administration (see Tables 2 and 3). Moreover, the mesor of heart rate (355 ± 3 versus 367 ± 4 beats/min for saline versus nicorandil, respectively) as well as the maxima of heart rate (464 ± 5 versus 511 ± 5 beats/min for saline versus nicorandil, respectively) were increased ($P < 0.05$) with nicorandil treatment. Finally, the post-treatment period indicated a significantly decreased mesor (322 ± 2 beats/min), maxima (438 ± 6 beats/min) and minima (259 ± 1 beats/min) as well as a significantly increased amplitude (37 ± 2 beats/min) of heart rate when compared to those recorded during nicorandil administration (367 ± 4, 511 ± 5, 280 ± 4 and 24 ± 2 beats/min for mesor, maxima, minima and amplitude of heart rate, respectively). No modifications of mesors, maxima, minima and amplitudes of body temperature or locomotor activity were elicited by nicorandil treatment.

Table 3 illustrates significant 2 and 7 h shifts in peaks of locomotor activity and heart rate, respectively, following nicorandil treatment (04.12 ± 0.20 versus 02.09 ± 0.42 h min for locomotor activity and 07.23 ± 0.16 versus 24.26 ± 0.23 h min for heart rate for nicorandil versus saline, respectively). The acrophase of body temperature was not significantly modified by nicorandil treatment. Finally, the acrophases of heart rate and locomotor activity, which were phase-shifted during nicorandil treatment, were restored during the post-treatment period.

4. Discussion

The major findings of this study include the deterioration of the diurnal rhythmicity of heart rate elicited by nicorandil treatment as a result of an increase in heart rate. Hence we conclude that during nicorandil treatment either no diurnal rhythmicity was detected in the heart rate or, if rhythmicity was detected, the acrophase of the heart rate was phase-shifted. Furthermore perturbations of the diurnal rhythms of body temperature, heart rate and locomotor activity could be observed after surgical implantation and oral drug administration via a tube placed into the stomach of the animals.

The perturbation of the diurnal rhythms of body temperature, heart rate and locomotor activity, which was ob-

served after the implantation of the transmitters, was induced by the surgical stress as well as by the ether anaesthesia. These data agree with the reported observations of Prudian et al. (1997), who showed a loss of the daily rhythms of body temperature, heart rate and locomotor activity after surgical implantation, related to both surgical procedures and anaesthesia. Furthermore, Drijfhout et al. (1995) reported that, in male rats, the surgical implantation of microdialysis probes under choral hydrate anaesthesia modified the circadian rhythm in body temperature and locomotor activity. Also Harper et al. (1996) have shown the deterioration of circadian rhythms following surgery and social stress.

The observed modifications of the mesors, maxima, minima, amplitude and acrophase of the diurnal rhythms of heart rate provoked by saline administration are consistent with the results of Kramer et al. (1993), who reported an increased heart rate caused by stress.

The repeated nicorandil treatment induced a loss of the diurnal rhythmicity of heart rate, an effect which was not observed during saline administration (Table 1) and which was therefore essentially due to the pharmacological action of nicorandil. This action is also reflected by the increased mesor and maxima of the heart rate (Table 2) as well as by the phase-shifted acrophases of heart rate and locomotor activity (Table 3) observed during nicorandil treatment. The observation that, in contrast to the mesor and maxima of the heart rate, the amplitude of the heart rate was not significantly modified by nicorandil can be explained by the fact that the amplitude was not simply assessed as half the difference between the maxima and minima, but as half the difference between the peak and trough of the fitted cosine wave calculated by a least-square cosine regression. Thus amplitude and acrophase were related to the fitted function and were not simply related to the studied phenomenon (De Prins and Hecquet, 1992). Nicorandil treatment provoked a short-lasting increase in heart rate (Fig. 1) during the resting phase (light phase) of the animals (during which heart rate is low). As the observed increase in heart rate was therefore most probably smoothened by the least-square cosine regression, it was of interest to consider the maxima and minima of the heart rate, which were registered raw data. In this study the time of drug administration was kept constant. Therefore the effects of the K^+ channel agonist on the diurnal rhythms and not the influence of its time of administration, were studied. As nicorandil was administered during the resting phase (light period) of the animals, it must be considered, as shown by many chronobiological studies of other drugs (Bruguerolle, 1992; Lemmer, 1992), that a different time of administration could have modified the diurnal rhythmicity in a different way.

Finally, as Fig. 1 and Tables 1–3 indicate, as soon as nicorandil treatment was stopped, the diurnal rhythmicity was re-established, the mesor, maxima and minima of heart rate were decreased, the amplitude of heart rate was increased and the acrophases of heart rate and locomotor activity, which were phase-shifted under nicorandil, were restored. Thus, nicorandil did not have any long-lasting effects on the diurnal rhythmicity of heart rate, which is consistent with the kinetic properties of nicorandil. Bachert et al. (1994) reported a rapid elimination of nicorandil administered to rats ($t_{1/2} = 2.5$ h for a 10 mg/kg i.v. nicorandil administration). Moreover, there was a significant decrease in the amplitude of the heart rate due to handling stress during oral administration. Although there was no significant difference, the amplitude of the heart rate seemed to continue to decrease when nicorandil was administered. As soon as nicorandil administration was stopped and with it the handling stress, the amplitude returned to its basal level. Amplitude and circadian rhythmicity are correlated: the lower the amplitude, the lower the possibility that there is circadian rhythmicity. As reported by Morgan and Minors (1995), the cosinor analysis describes, by means of a statistical test, whether the amplitude of the fitted cosine curve is significantly different from zero. It gives therefore a probability of the data being better described by a cosine curve than by a straight line. The increase in heart rate during nicorandil treatment, which happened when heart rate was low, could result in a smoothening of the fitted cosine wave and therefore be responsible for the lower amplitude. This could explain the loss of the diurnal rhythmicity of heart rate in 3 animals, as revealed by Fourier analysis.

In addition, the diurnal rhythmicity of body temperature was not modified by either the stress of oral administration or the repeated nicorandil treatment. The diurnal rhythm in body temperature is known to be a marker of circadian rhythmicity in rats (Gordon, 1994a). Therefore, although heart rate and locomotor activity also follow circadian patterns in rats (Meinrath and D'Amato, 1979), the observed modifications in the diurnal rhythmicity of heart rate were not able to influence on the diurnal rhythmicity of body temperature.

In conclusion, this study showed that the repeated administration of nicorandil altered the diurnal rhythmicity of heart rate in rats while the diurnal rhythmicity of body temperature and locomotor activity was not modified (except for the phase-shifted acrophase of locomotor activity). The observed modifications of heart rate were restored as soon as nicorandil was stopped. Future studies will investigate whether different times of drug administration will shift the acrophase of the heart rate to a similar extent and independently of the circadian phase, and thus may influence the diurnal rhythms of body temperature, heart rate and locomotor activity in rats.

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