Spectral analysis of heart rate variability of lizard, *Gallotia galloti*

JULIAN GONZALEZ GONZALEZ AND LUIS DE VERA PORCELL
Department of Physiology, Medical Faculty, University of La Laguna, Teneriffe, Canary Islands, Spain

GONZALEZ GONZALEZ, JULIAN, AND LUIS DE VERA PORCELL. *Spectral analysis of heart rate variability of lizard, Gallotia galloti.* Am. J. Physiol. 254 (Regulatory Integrative Comp. Physiol. 23): R242–R248, 1988.—The beat-to-beat heart rate of the lizard, *Gallotia galloti*, at rest shows short-term oscillations, the frequency of which varies with body temperature. Spectral analysis of the heart rate variability signal shows that, above 20°C, two major frequency components are present: the first component has a mean frequency ranging from 0.032 at 20°C to 0.070 Hz at 35°C and the second from 0.039 at 20°C to 0.10 Hz at 35°C of body temperature. The beat-to-beat heart rate variability does not seem to be correlated with ventilatory activity. The two spectral components could be associated as in mammals with the activity of the control systems that regulate the circulation, especially with the cutaneous vasomotor thermoregulatory and endogenous pressure vasomotor activities. Transient interactions between both components are described.

heart rate variability signal; oscillations; reptile

THE HEART RATE VARIABILITY (HRV) of mammals (beat-to-beat variation of their cardiac period or frequency) has long been known to arise from respiratory activity. The human HRV has been attributed mainly to cardiovascular reflexes related to circulatory control, integrated patterns of cardiovascular response to a variety of functional demands such as exercise or thermal regulations, and the intrinsic mechanisms that are the source of cardiac rhythmicity (19). On the other hand, the heart rate variability signal (HRVS) (real time representation of the instantaneous heart rate) shows, in humans at rest, short-term oscillations that have been attributed to the activity of physiological control systems. The power spectrum of the different segments of this signal shows three frequency components, the first attributed to thermal vasomotor activity at ~0.05 Hz, the second to the blood pressure control system activity at ~0.10 Hz, and the third to respiratory arrhythmia in the range of 0.25 Hz (13, 25). The thermal vasomotor component has been shown to be entrained by external periodic thermal stimuli (15, 16); the pressure control component also presents the phenomenon of frequency-selective entrainment (12). These components are not stable throughout the sequential recordings of HRVS, showing amplitude and frequency variations attributed to transient interaction between the different components (17). Studies in humans (21) and in the conscious dog (1) show that low-frequency fluctuations in heart rate (below 0.1 Hz) are jointly mediated by the β-sympathetic and parasympathetic nervous systems, whereas higher frequency fluctuations are mediated only by the parasympathetic system.

The nature of the beat-to-beat HRV of reptiles and the factors contributing to it have not been studied previously. It is only known that the mean heart rate of reptiles varies with temperature, size, metabolism, respiratory state, and other external nonspecific factors (28). In particular, a greater mean heart rate during active ventilation than during apnea has been reported (11). On the other hand, it has been shown that central control neural mechanisms affecting the cardiovascular functions are present in reptiles (4, 28). It could thus be that in reptiles, as in mammals, the instantaneous heart rate is affected by the activity of physiological control systems that regulate circulation through the autonomic nervous system.

The aim of investigating the HRV of the lizard, *Gallotia galloti*, by means of spectral analysis is to ascertain whether definite frequency components similar to those present in the HRVS of mammals exist in reptiles. The influence of body temperature and respiratory activity on the spectral content of the HRVS of these animals is also studied. Finally, the possible relations between the frequency components of the HRVS power spectrum and the dynamics of the cardiovascular control systems described in reptiles are discussed with a view to contributing to the knowledge of the neural control of reptilian circulation.

MATERIALS AND METHODS

Six lizards, *G. galloti*, captured in Teneriffe (Canary Islands, Spain) weighing from 34 to 75 g and of undefined sex were used as experimental animals. The animals were kept in terraria where ambient temperature ranged from 19 to 22°C and relative humidity from 50 to 60%. Water and food, consisting of small pieces of banana and tomato, were provided ad libitum.

In six animals, bipolar electrocardiogram (ECG) was recorded at seven monitored temperature stations in the range of 5–35°C, by two small stainless steel plate electrodes inserted below the skin to the right and left of the lizard's dorsal region, one anterior and the other posterior to the heart. A third electrode situated in the caudal region near the tail served as an earth electrode.

In four lizards, simultaneous recording of ventilatory activity and ECG was carried out. The ventilatory activ-

ity was recorded by means of an active stainless steel needle electrode implanted into the nasal membrane and a stainless steel screw reference electrode implanted in the parietal bone (6).

Recordings were made in two identical noiseless chambers (40 × 40 × 30 cm) in one of which the ambient temperature could be thermostatically controlled at between 5 and 20 ± 0.5°C and in the other at between 25 and 35 ± 0.5°C. Relative humidity in both chambers ranged from 35 to 40%. The chamber floors were covered with cork, and the lizards were free to run about on it. In all experiments the animal was kept within the chamber for a 24-h habituation period, after which measurements were made when the animal was at rest.

Core body temperature was monitored by an electrical thermometer (Nihon-Kohden MGA III-219) provided with a small thermistor probe that was inserted into the animal's cloaca 2 cm deep. The thermistor leads were attached to the tail.

The signals from the lizards were fed into a recording system (Nihon-Kohden polygraph) that was connected to an automatic data acquisition and processing system for small animals developed in our laboratory and based on a microcomputer (7).

Voltages from the polygraph were fed into the system's analog-digital converter and handled by software. The measurements of the consecutive R-R intervals of the lizard ECG were made by a BASIC program and an ASSEMBLER subroutine that provided us with on-line ECG peak R detection and, consequently, the magnitude calculation of the consecutive R-R intervals. Approximately 600 R-R intervals were recorded at the seven body temperatures for each lizard. An ASSEMBLER program was used for simultaneous sampling of the ECG and the ventilatory activity signals; sampling rate was 500 Hz for the ECG signal and 250 Hz for the other. Samples at each body temperature were stored on diskette. Off-line measurements of the consecutive R-R intervals from the ECG-sampled signals were made by use of an algorithm similar to that used in the on-line operation.

An instantaneous heart rate variability signal was obtained by interpolating the inverse of the R-R interval at RR/100-ms sample periods between every two R wave occurrence times. Then, for power spectra analysis purposes, this signal was sampled at 0.25, 0.50, 0.50, 1, 2, 2.5, and 5 Hz at body temperatures of 5, 10, 15, 20, 25, 30, and 35°C, respectively. The data window lengths at these temperatures were 512, 256, 256, 128, 128, 102.4, and 51.2 s, respectively. The ventilatory signal was sampled at the same rates as HRVS and equivalent window lengths were used for the different body temperatures.

The circular autocorrelation function of the sampled HRVS was calculated and plotted at different body temperatures. The power spectra density function of the HRV and of the ventilatory sampled signals were estimated from the Fast Fourier Transform (FFT) algorithm. Previous to this operation, the mean value of each signal segment was calculated and subtracted from each data point. Each signal segment was then cosine tapered over the first and last 10% of the samples. Confirmation that some spectral bands of the HRVS spectrum could be distinguished from white noises was obtained by the Kolmogorov-Smirnov test (14, 25). The peak frequency of the main frequency bands from the individual spectra at a given body temperature was noted and then averaged. The mean power of the frequency bands selected was obtained from the ensemble average of the individual spectra. To reduce interanimal variance, power spectrum coefficients were normalized with respect to total power. Mean power values for the bands at different body temperatures were plotted together with the 95% level of significance (3). Mean peak frequency and mean power of the frequency bands were fitted to exponentials and straight lines, respectively, by use of the least-squares method.

Sequential or running spectra of HRVS at a given body temperature were made by repeatedly stepping up the window length by 50 s.

RESULTS

Figure 1 shows segments of HRVS at three body temperatures in which short-term fluctuations can be observed. The autocorrelation functions of HRVS (Fig. 2) show the existence of periodicities distinguishable from noise at the different body temperatures studied. The data window lengths selected at each body temperature for the spectral analysis of the HVRSs were chosen after inspection of the autocorrelation functions. Moreover, the Kolmogorov-Smirnov test, applied to the cu-

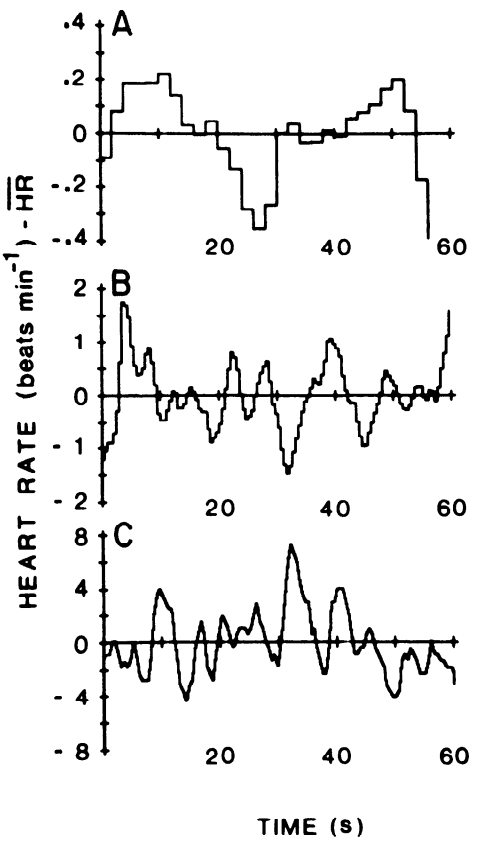

FIG. 1. Instantaneous heart rate variability signals at 15 (*A*), 25 (*B*), and 35°C (*C*) of body temperature. Heart rate values minus mean heart rate corresponding to segment plotted are represented in ordinate.

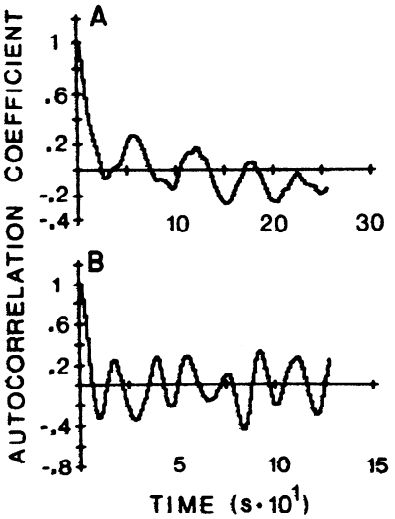

FIG. 2. Circular autocorrelation functions of 2 heart rate variability signals at 10 (*A*) and 20°C (*B*) of body temperature.

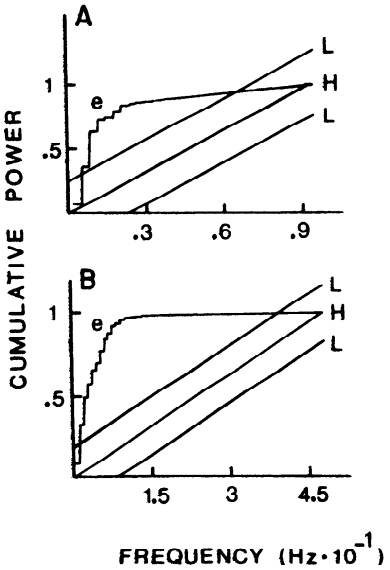

FIG. 3. Cumulative normalized spectral power (*e*) of 2 heart rate variability signals recorded at 10 (*A*) and 25°C (*B*) of body temperature. Lines (L) are critical limits plotted for a $P = 0.05$ level by use of hypothesis of uniform spectral power density (H) from Kolmogorov-Smirnov test.

mulative normalized power spectra (Fig. 3), showed in most cases the existence of some nonrandom spectral components in the HRVS spectra at the different body temperatures studied. The frequency band occupied by these nonrandom components ranged from 0.008 to 0.150 Hz.

The power spectra of HRVS at low body temperatures (5–15°C) exhibit only one main component, although in some recordings (see Fig. 1 at 15°C) a higher frequency component having only a slight spectral representation can be noted. Above 20°C two spectral components can be distinguished (see Fig. 4). The mean peak frequency and the ranges of these spectral components are shown in Fig. 5. The peak frequencies of these bands increase exponentially ($P < 0.01$) with body temperature.

Two frequency bands (1 between 0.008 and 0.075 and the other between 0.075 and 0.150 Hz) were selected to compare the power of the spectral components of the HRVS at different body temperatures, because the peak frequency of these components falls within these bands. The power in these bands expressed as a percentage of the total spectral power is shown in Fig. 6. The relative power density of the first frequency band decreases linearly with body temperature ($P < 0.01$), whereas that of the second frequency band increases linearly ($P < 0.01$). No significant differences ($P < 0.05$) are found between the relative mean power of the two frequency bands at body temperatures of 30 and 35°C.

Table 1 shows the mean values of respiratory and heart rates at the different body temperatures studied. Simultaneous recordings of the ECG and ventilatory activity do not show changes in the R-R interval duration associated with the inspiratory and expiratory phases of the ventilatory cycle. In fact, no respiratory component appears in the power spectrum of the HRVS of this lizard at the different body temperatures investigated. Indeed, the frequency band occupied by the ventilatory activity in the power spectrum of the ventilatory signal lies far away from the frequency range taken up by the oscillatory components of the HRVS (Fig. 7).

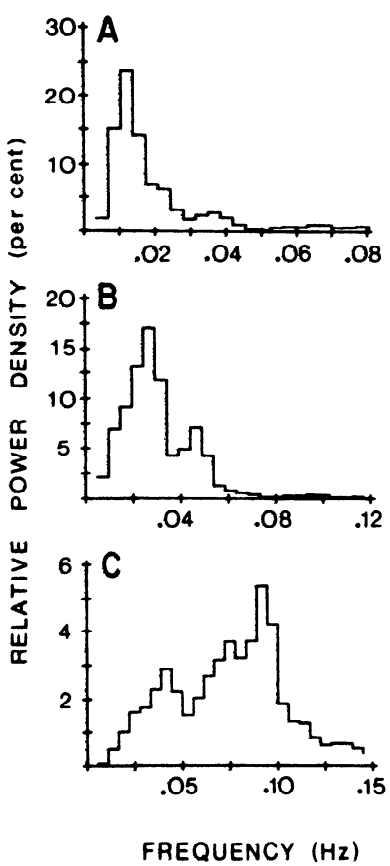

FIG. 4. Power spectra of heart rate variability signals at 10 (*A*), 20 (*B*), and 30°C (*C*) of body temperature from 44-g lizard.

The two spectral components of the HRVS that can be distinguished above 20°C of body temperature are not always well defined throughout the recording corresponding to a given body temperature but present transient variations in their power content and peak frequency. These features are shown in Fig. 8, which pre-

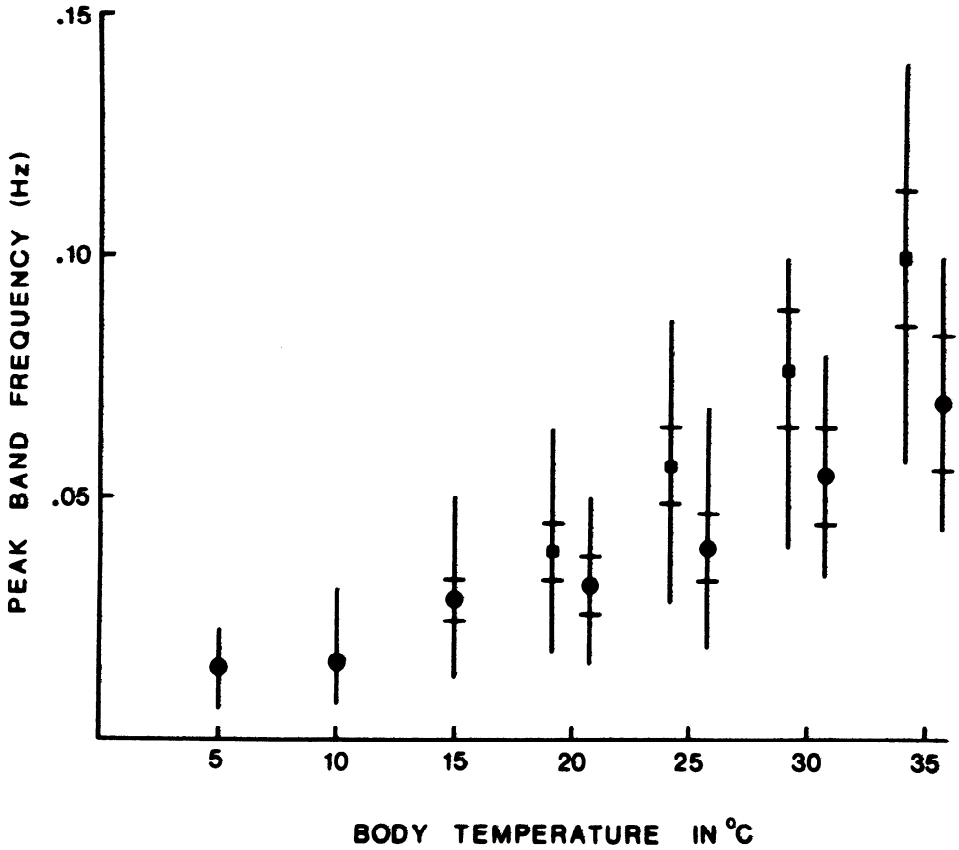

FIG. 5. Relationship between peak frequency of 0.008- to 0.075 (■)- and 0.075- to 0.150-Hz (●) spectral bands and body temperature. |, Ranges and —, ± 2 SE. To avoid overlap, peak frequency values at 20, 25, 30, and 35°C have been displaced to right and left of each temperature studied.

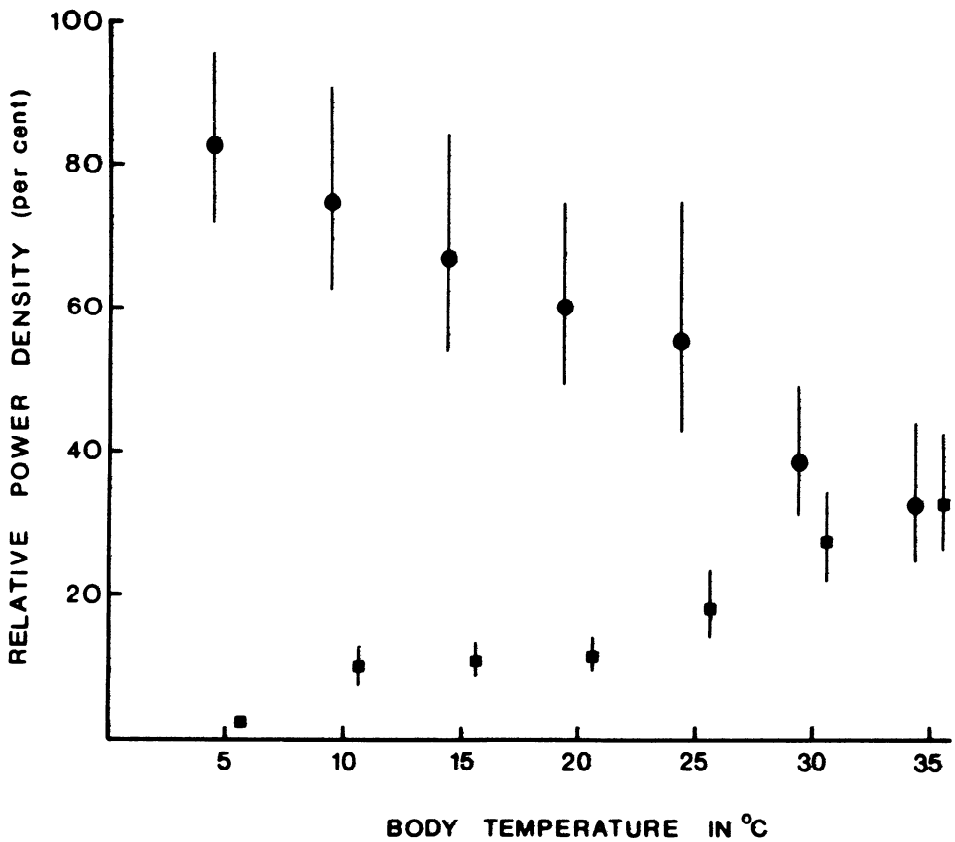

FIG. 6. Relationship between relative power density of 0.008- to 0.075 (●)- and 0.075- to 0.150-Hz (■) spectral bands and body temperature. |, 95% Confidence interval. To avoid overlap, power density values are displaced to right and left of each body temperature.

sents sequential spectra of HRVS at 30°C. In Fig. 8*A* both the low- and the high-frequency spectral components are clearly represented with the peak frequency of the higher spectral component being double that of the lower. In the following sequential HRVS spectra, it can be seen that the relative power of the lower component decreases, whereas that of the higher component increases (Fig. 8, *B–D*) to the point at which only the

TABLE 1. *Mean values of respiratory and heart rate*

BT, °C	RR, breaths/min	HR, beats/min
5	8.2±1.3	6.7±0.3
10	10.9±1.1	13.6±0.7
15	16.3±3.1	23.0±1.7
20	23.6±3.2	39.3±3.8
25	33.9±4.4	65.4±3.9
30	39.8±4.5	92.6±5.6
35	60.0±5.5	130.6±7.1

Values are means ± SE for 6 animals. BT, body temperature; RR, respiratory rate; HR, heart rate.

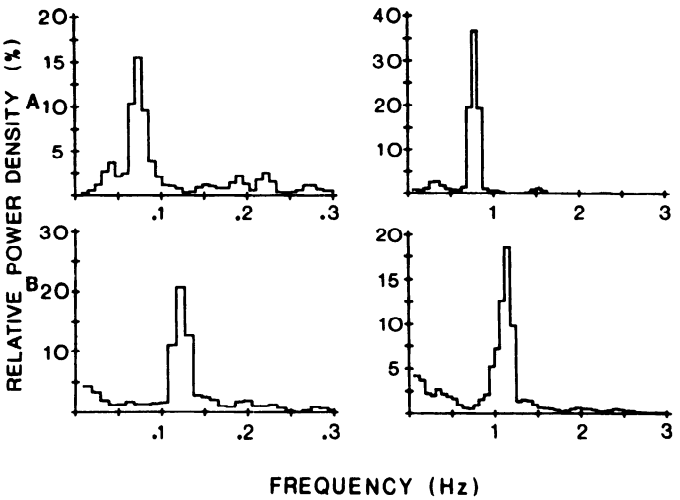

FIG. 7. Power spectra of heart rate variability signal (*left*) and ventilatory signal (*right*) at 25 (*A*) and 35°C (*B*) of body temperature.

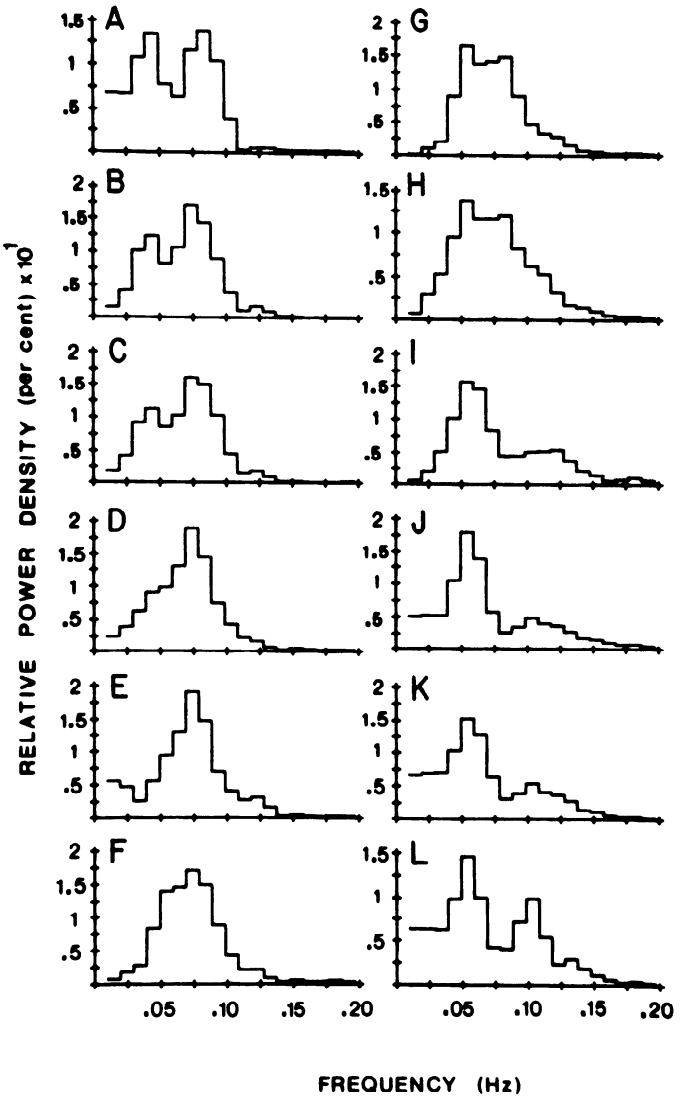

FIG. 8. Sequential power spectra of heart rate variability signal at 30°C of body temperature in 58-g lizard showing changes of 2 components.

higher component appears to be present (Fig. 8*E*). In this situation, it seems that the lower component transiently switches off, whereas only the higher component oscillates. In the following sequential spectra, a reverse situation arises in which the higher component decreases in power until it almost disappears with only the lower component appearing (Fig. 8, *F–I*). Finally, the initial situation is again reached, where the two components are evident, with the peak frequencies maintaining the same relation (Fig. 8, *J–L*).

DISCUSSION

The beat-to-beat heart rate of the lizard *G. galloti* at rest shows a variability that is characterized, in its time course representation, by short-term fluctuations distinguishable from noise and whose oscillatory frequencies increase with body temperature.

In HRV studies, spectral analysis has been used to investigate the short-term fluctuations exhibited by the sequential instantaneous heart rate values to reveal the activity of various underlying physiological processes (1, 13, 25). For this purpose, a HRVS is first generated from the event series representing R waves of ECG, and then the power spectrum density function is generally estimated via FFT algorithm after equidistant sampling of the signal (5, 18). It has been shown, however, that the bandwidth of whatever HRVS is chosen is less than about half the mean heart rate (24), so that the choice of sampling rates greater than the mean heart rate will prevent spectral aliasing. Because the mean heart rate values of our animals vary with body temperature, the sampling rate at each temperature is chosen in accordance with the above criterion. Furthermore, due to the fact that the oscillatory frequency exhibited by the HRVS increases with the body temperature, the data window length for spectral analysis is selected to obtain a suitable spectral resolution at the different temperatures.

The spectral analysis of the HRVS shows that below 20°C in most of the animals only one spectral component is discernible, its mean frequency ranging from 0.015 at 5°C to 0.029 Hz at 15°C. In some recordings, at these body temperatures, a second oscillation of higher frequency but lower amplitude appears superimposed on the first; this oscillation barely appears in the power spectrum. Above 20°C, the two oscillations are clearly distinguishable in the power spectra of the HRVS, reaching the same relative power density at 35°C. The mean frequency of the slower oscillation ranges from 0.032 at

20°C to 0.076 Hz at 35°C, and the higher frequency oscillation varies from 0.039 to 0.10 Hz in the same range of body temperatures. We did not find in the literature any report describing this kind of oscillation in reptiles. Only oscillations in the mean heart rate (mean values normally calculated over periods greater than 1 min) are described in the report on *Caiman* (10, 11). No spectral component was distinguishable around the ventilatory frequency of these animals; it thus appears that the beat-to-beat cardiorespiratory synchrony present in mammals does not occur in our reptiles. On the other hand, the frequency of the two spectral components appearing in the HRVS of our lizards above 25°C is close to that of the first two spectral components observed in the power spectra of the resting human HRVS (13, 16, 25). The slower frequency (temperature component) is related to the dynamics of the peripheral thermal vasomotor control system, and the higher frequency (pressure component) to the operation of the blood pressure control system.

It is known that in reptiles the blood pressure is centrally controlled by thermally sensitive mechanisms (23) and the thermal stimulation of the hypothalamus produces blood pressure changes accompanied by heart rate changes (22), effects absent below body temperatures of ~20°C (9). It thus appears probable that the higher frequency component in the HRVS of our animals arises, as does the pressure component of mammals, from the activity of the endogenous vasomotor activity within their blood pressure control system. Furthermore, this component almost disappears below 20°C, which agrees with previous reports on blood pressure change below this temperature.

Furthermore, physiological thermoregulatory mechanisms have been shown to exist in several reptilian species (2, 4, 8, 26) within which the cardiovascular mechanisms that are mainly involved have been proven to be changes in peripheral circulation and heart rate. Moreover, it has been shown that an increase in heart rate during peripheral heating occurs before changes in core temperature (20). Consequently, it has been suggested (27, 28) that such reflex adjustments may involve either cutaneous thermal receptor or baroreceptor mechanisms that are activated secondarily to the cutaneous vasodilation induced by heating. It therefore appears that the dermovascular temperature responses in reptiles are dependent on neural factors, and this thermoregulatory dermovasomotor activity affects the instantaneous heart rate as it does in humans, giving rise (as in humans) to the slow-frequency component that appears in the HRVS of our reptiles.

The individual spectral components mentioned are not always clearly defined throughout the HRVS recordings at a given body temperature, showing instead transient changes in their frequency and relative power density. In fact, sequential power spectral representation of the HRVS shows situations in which the slower component decreases in power, whereas the higher frequency component increases and at the same time its oscillatory frequency decreases, being displaced towards an intermediate frequency region. In other cases it is the higher frequency component that almost disappears with only the slower component remaining in the recording, although with a rather higher oscillatory frequency. These phenomena suggest the existence of a dynamic interaction between the two components and thus between the operations of the underlying physiological systems involved. A similar interaction between the blood pressure component and temperature component activity has been reported in the HRVS of humans (16).

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REFERENCES

1. AKSELROD, S., D. GORDON, F. A. UBEL, D. C. SHANNON, A. C. BARGER, AND R. J. COHEN. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science Wash. DC* 213: 220–222, 1981.
2. BARTHOLOMEW, G. A., AND V. A. TUCKER. Control of changes in body temperature, metabolism, and circulation by the agamid lizard *Amphibolurus barbatus*. *Physiol. Zool.* 36: 199–219, 1963.
3. BENDAT, J. S., AND A. G. PIERSOL. *Random Data: Analysis and Measurement Procedures*. New York: Wiley, 1971.
4. CRAWSHAW, L. I. Temperature regulation in vertebrates. *Annu. Rev. Physiol.* 42: 473–491, 1980.
5. DE BOER, R. W., J. M. KAREMAKER, AND J. STRACKEE. Comparing spectra of a series of points events, in particular for heart rate variability data. *IEEE Trans.* 31: 384–387, 1984.
6. DE VERA PORCELL, L., AND J. GONZÁLEZ GONZÁLEZ. Effect of body temperature on the ventilatory responses in the lizard *Gallotia galloti*. *Respir. Physiol.* 65: 29–37, 1986.
7. GONZÁLEZ GONZÁLEZ, J., AND L. DE VERA PORCELL. Sistema microcomputarizado de análisis de señales biológicas para su utilización en laboratorios de investigación animal. *Resumenes Conf. Iberoamer. Bioingen Asturias Spain*, 1984, p. 114.
8. GONZÁLEZ GONZÁLEZ, J., AND L. DE VERA PORCELL. Physiological thermoregulation of the Canary lizard *Gallotia galloti*. *Comp. Biochem. Physiol. A Comp. Physiol.* 83: 709–713, 1986.
9. HEATH, J. E., E. GASDORF, AND R. G. NORTHCUTT. The effect of thermal stimulation of anterior hypothalamus on blood pressure in the turtle. *Comp. Biochem. Physiol.* 26: 509–518, 1968.
10. HUGGINS, S. E., H. E. HOFF, AND R. V. PEÑA. Heart and respiratory rates in crocodilian reptiles under conditions of minimal stimulation. *Physiol. Zool.* 42: 320–333, 1969.
11. HUGGINS, S. E., H. E. HOFF, AND R. V. PEÑA. The respiratory-heart rate response in crocodilian reptiles. *Physiol. Zool.* 43: 10–18, 1970.
12. HYNDMAN, B. W. Cardiovascular recovery to psychological stress: a means to diagnose man and task? In: *The Study of Heart-Rate Variability*, edited by R. I. Kitney and O. Rompelman. Oxford, UK: Oxford Univ. Press, 1980, p. 191–224.
13. HYNDMAN, B. W., R. I. KITNEY, AND B. M. SAYERS. Spontaneous rhythms in physiological control systems. *Nature Lond.* 233: 339–341, 1971.
14. JENKINS, G. M., AND D. G. WATTS. *Spectral Analysis and Its Applications*. San Francisco, CA: Holden-Day, 1968.
15. KITNEY, R. I. An analysis of the nonlinear behaviour of the human thermal vasomotor control system. *J. Theor. Biol.* 52: 231–248, 1975.
16. KITNEY, R. I. An analysis of the thermoregulatory influences on heart-rate variability. In: *The Study of Heart-Rate Variability*, edited by R. I. Kitney and O. Rompelman. Oxford, UK: Oxford Univ. Press, 1980, p. 81–106.
17. KITNEY, R. I., T. FULTON, A. H. MCDONALD, AND D. A. LIKENS. Transient interactions between blood pressure, respiration and heart rate in man. *J. Biomed. Eng.* 7: 217–224, 1985.
18. LUCZAK, H., AND W. LAURING. An analysis of heart rate variability. *Ergonomics* 16: 85–97, 1973.
19. MCDONALD, A. H. Mechanisms affecting heart-rate. In: *The Study of Heart-Rate Variability*, edited by R. I. Kitney and O. Rompelman. Oxford, UK: Oxford Univ. Press, 1980, p. 3–12.
20. MORGAREIDGE, K. R., AND F. N. WHITE. Cutaneous vascular

changes during heating and cooling in the Galapagos marine iguana. *Nature Lond.* 223: 587–591, 1969.
21. POMERANZ, B., R. J. B. MACAULAY, M. A. CAUDILL, I. KUTZ, D. ADAM, D. GORDON, K. M. KILBORN, A. C. BARGER, D. C. SHANNON, R. J. COHEN, AND H. BENSON. Assessment of autonomic function in humans by heart rate spectral analysis. *Am. J. Physiol.* 248 (*Heart Circ. Physiol.* 17): H151–H153, 1985.
22. RODBARD, S., F. SAMSON, AND D. FERGUSON. Thermosensitivity of the turtle brain as manifested by blood pressure changes. *Am. J. Physiol.* 160: 402–408, 1950.
23. RODBARD, S., M. TINSLEY, H. BORNSTEIN, AND L. TAYLOR. Role of the central nervous system in the body temperature-arterial pressure relationship. *Am. J. Physiol.* 158: 135–140, 1949.
24. ROMPELMAN, O., A. J. R. M. COENEN, AND R. I. KITNEY. Measurement of heart-rate variability: part I.–comparative study of heart-rate variability analysis methods. *Med. Biol. Eng. Comput.* 15: 233–239, 1977.
25. SAYERS, B. M. Analysis of heart rate variability. *Ergonomics* 16: 17–32, 1973.
26. SMITH, E. N., E. A. STANDORA, AND S. L. ROBERTSON. Physiological thermoregulation of mature alligators. *Comp. Biochem. Physiol. A Comp. Physiol.* 77: 189–193, 1984.
27. WHITE, F. N. Temperature and the Galapagos marine iguana. Insights into reptilian thermoregulation. *Comp. Biochem. Physiol. A Comp. Physiol.* 45: 503–513, 1973.
28. WHITE, F. N. Circulation. In: *Biology of the Reptilia,* edited by C. Gans and W. R. Dawson. New York: Academic, 1976, vol. 5, p. 275–334.