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Effect of Circadian Rhythm on the Neuronal Uptake and Sensitivity to the Inotropic Action of Norepinephrine by Rat Atria

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MEHRABANI, P. AND J. R. BASSETT. *Effect of circadian rhythm on the neuronal uptake and sensitivity to the inotropic action of norepinephrine by rat atria.* PHARMACOL BIOCHEM BEHAV 32(2) 475–477, 1989.—The circadian rhythm for plasma corticosterone was determined. Animals were then killed at times corresponding to high and low periods of the circadian rhythm in plasma corticosterone. Myocardial sensitivity to norepinephrine was measured at these time periods as the ED_{50} of the catecholamine, obtained using electrically driven rat atria. The uptake of ^{3}H-norepinephrine by spontaneously beating atria was also measured at both time periods. A circadian variation in the uptake of ^{3}H-norepinephrine by the rat atria was observed. This variation in uptake was associated with a variation in plasma corticosterone, but was not associated with any change in myocardial sensitivity to norepinephrine.

Neuronal uptake Myocardial sensitivity Norepinephrine ACTH Circadian rhythm

BASSETT, Strand and Cairncross (2) reported that (1–24)adrenocorticotrophin [(1–24)ACTH] potentiated the myocardial sensitivity to norepinephrine in a dose-dependent manner. Since a similar enhanced myocardial sensitivity following exposure to stress had been attributed to an inhibition of the neuronal reuptake process (uptake 1) (1), it was postulated that (1–24)ACTH may exert its action on the heart by a similar inhibition of norepinephrine uptake (2). Indeed, it has been now shown that (1–24)ACTH can inhibit the neuronal uptake of norepinephrine in rat atria in vitro at relatively low bath concentrations (13). While the actual bath concentrations used by Mehrabani and Bassett (13) were initially within the range reported for plasma ACTH following exposure to a stressor (7,8), at the end of the incubation period the actual bath concentrations of (1–24)ACTH were similar to the peak plasma ACTH levels reported to occur during the circadian rhythm (12, 14, 15).

There have been numerous studies showing that there is a daily rhythm in adrenal responsiveness to ACTH, possible due to diurnal changes in ACTH receptor affinity or coupling with adenylate cyclase (10). The adrenal rhythm is not dependent on, but is in phase with, the circadian rhythm in plasma ACTH levels (10); the adrenal cortex being more sensitive to ACTH during the more active portion of the circadian rhythm (4). If the same diurnal variation in adrenal responsiveness to ACTH applies to the extra-adrenal actions of ACTH, such as those postulated for cardiac tissue (13), then the circadian variation in circulating ACTH levels may be associated with a circadian variation in the neuronal reuptake of norepinephrine, and a resulting variation in the myocardial sensitivity to the catecholamine.

The present study was undertaken to investigate this question.

METHOD

Animals

Male CSF rats, 90±5 days old, were used in all experiments. The animals were housed in groups of 3 under conditions of constant temperature and humidity (21±0.5°C, 46% humidity), and subjected to a 12-hr reversed night-day schedule (light 20.00 to 08.00 hr) beginning at least 14 days prior to the commencement of experimentation. Food and water were provided ad lib.

Circadian Variation in Plasma Corticosterone

Measurement of ACTH levels is generally associated with serious technical difficulties. These mainly relate to the rapid destruction of ACTH by a wide variety of plasma enzymes, the dissociation of biological and immunological activity, and the loss of ACTH due to its extraordinary propensity to bind to inert materials (5). On the other hand, corticosterone is a much more stable hormone, and the methods for its analysis are generally more robust and easily conducted. For this reason, since changes in plasma corticosterone concentration have been shown to closely reflect changes in plasma ACTH (12), plasma corticosterone concentrations instead of

TABLE 1

VARIATION IN MYOCARDIAL SENSITIVITY TO NOREPINEPHRINE AND THE UPTAKE OF ^{3}H-NOREPINEPHRINE (NE) BY SPONTANEOUSLY BEATING RAT ATRIA AT TWO TIME PERIODS OF THE DAY

Time of Day (hr)	Myocardial Sensitivity ED_{50} ($\times 10^{-8}$ M) (mean ± S.E.M.)	Uptake of ^{3}H-NE DPM/mg Tissue (mean ± S.E.M.)
09.00–11.00 (lights off)	4.4 ± 0.5 (10)	5731 ± 169 (12)
20.00–22.00 (lights on)	4.7 ± 0.6 (7)	6335 ± 172 (12)
t-test; p	>0.05	<0.02

Number in parentheses=number of animals/group.

ACTH concentrations were measured over a 26-hr period in order to determine the high and low periods of the circadian variation in circulating ACTH. Six animals were killed on the hour every 2 hours over the 26-hr period. Animals were removed from their home cage in the animal holding room with as little disturbance as possible to the remaining animals. The animals were then killed immediately by cervical dislocation and exsanguinated. The blood was collected in heparinized tubes and centrifuged in order to obtain cell free plasma which was then frozen. Plasma corticosterone levels were assayed subsequently by a competitive protein-binding method (9), and the peak and trough periods of the circadian pattern determined.

Circadian Variation in Myocardial Sensitivity and Uptake

For both the measurement of myocardial sensitivity to norepinephrine and the uptake of ^{3}H-norepinephrine, tissues were obtained at times corresponding to high (09.00–11.00 hr) and low (20.00–22.00 hr) periods of the circadian variation in plasma corticosterone, as determined above. The results obtained for the two time periods were compared using a t-test for two independent samples. Myocardial sensitivity and uptake were determined on different groups of animals, the numbers in each group shown in Table 1.

Myocardial sensitivity. The technique was similar to that described by Bassett, Strand and Cairncross (2). Left atrial segments, suspended in Krebs-Henseleit solution maintained at 32.5°C, were stimulated with a DC pulse at a frequency of 1 Hz delivered through a punctate platinum electrode. Contraction amplitude was recorded using a Cardiotrace recorder. Cummulative log dose-response curves for norepinephrine were obtained in triplicate. Tissue responses were expressed as a percentage of the maximal response determined for each curve. Regression lines were fitted to the linear portions of the curves by the method of least squares and the ED_{50} for norepinephrine calculated using the regression coefficients. The mean ED_{50} was used as a measure of myocardial sensitivity to the catecholamine.

Uptake of ^{3}H-norepinephrine. The method was essentially that described by Bassett and Cairncross (1). Spontaneously beating atria were suspended in an organ bath containing Krebs-Henseleit solution and maintained at 29°C. The atria were incubated with L-[7,8-^{3}H]norepinephrine (2.79×10^{-7} M) for a period of 7 min. This concentration of norepinephrine had previously been determined as the ED_{50} for noradrenaline on spontaneously beating atria at 29°C. The ^{3}H-norepinephrine (specific activity 41.4 Ci/mmole) was obtained chromatographically pure from Amersham International. Following incubation with the labelled amine, the atria were removed from the organ bath, washed in Krebs-Henseleit solution and blotted. The tissues were weighed, then digested in 1.0 ml of HCS tissue solubilizer (Amersham) at 50°C overnight. The samples were counted in a Packard scintillation counter. Total tissue radioactivity was calculated as disintegrations/min/mg of atrial tissue.

RESULTS

The circadian variation in plasma corticosterone concentration is shown in Fig. 1. A one-way analysis of variance showed a significant variation in corticosterone concentration over the 26-hr period studied, $F(13,70)=10.65$, $p<0.01$. Testing of the individual means using the Tukey test showed the critical value for a significant difference between any two means to be 10.7 μg/100 ml at the 5% level and 12.4 μg/100 ml

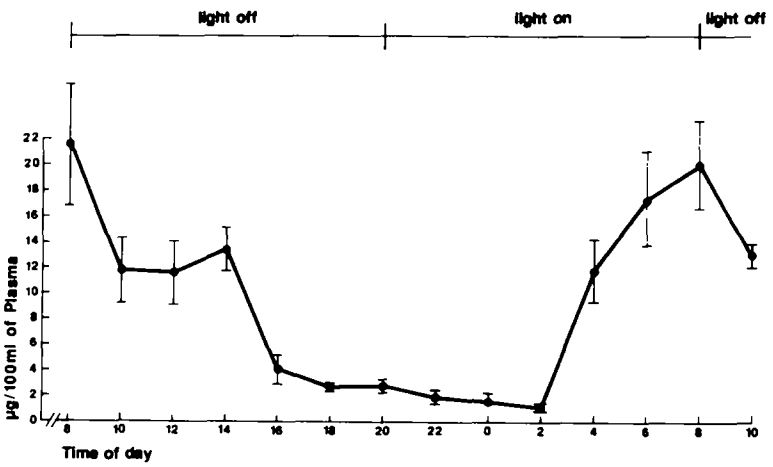

FIG. 1. Circadian rhythm of plasma corticosterone concentration over a 26-hr period. Each point represents the mean of 6 animals. Vertical bars designate ±S.E.M. Samples were taken every two hours. The periods of light are indicated by the top line.

at the 1% level (MS_{error}=29.1). The plasma corticosterone concentration had risen significantly by 04.00 hr (4 hr before lights off) and remained significantly elevated between the hours of 06.00 and 14.00. By 16.00 hr the plasma corticosterone levels had fallen, remaining at a low level until 02.00 hr then rising again by 04.00 hr. On the basis of this observed rhythm the two time periods chosen for subsequent study were 09.00 to 11.00 hr (a period of activity when the lights were off) and 20.00 to 22.00 hr (a period of inactivity when the lights were on).

The myocardial sensitivity to norepinephrine and the uptake of ^{3}H-norepinephrine by the rat atria, at the two chosen time periods, are shown in Table 1. There was no significant difference (unpaired t-test; $p>0.05$) in the ED_{50}s determined at either time period (lights off, 09.00 to 11.00 hr, and lights on, 20.00 to 22.00 hr). However, the uptake of ^{3}H-norepinephrine was significantly less in the time period associated with the lights off than it was in the time period associated with the lights on ($p<0.02$; unpaired t-test).

DISCUSSION

Allowing for differences in light period and activity times, the circadian pattern in plasma corticosterone seen in this study is similar in form to that reported by other researchers (3, 6, 16); there being a gradual rise in the glucocorticoid concentration beginning mid-light phase and peaking at the onset of dark, followed by a gradual decline during the dark period. While plasma ACTH levels were not measured, it is reasonable to assume that periods of high plasma corticosterone will also be periods of high plasma ACTH, and similarly low plasma steroid will be associated with low plasma ACTH. The assumption can be made since ACTH is the hormone responsible for the release of corticosterone from the adrenal gland, and a close association between the plasma concentrations of the two hormones has previously been reported (12).

The present study demonstrates that there is a circadian variation in the uptake of ^{3}H-norepinephrine by the rat atria; there was a significant reduction in the level of uptake in the period associated with high plasma ACTH concentration (09.00 to 11.00 hr) when compared with the period of low plasma ACTH (20.00–22.00 hr). This finding is consistent with the observation that (1–24)ACTH at low doses can inhibit the neuronal uptake of norepinephrine by rat atria in vitro (13). The inhibition of uptake, however, was not associated with any change in sensitivity. The myocardial sensitivity to norepinephrine, as measured by the ED_{50}, did not vary between the two time periods. It has been proposed that the enhanced myocardial sensitivity seen in vitro with (1–24)ACTH was due to the ability of this hormone to inhibit norepinephrine reuptake (13). Since the neuronal reuptake of the catecholamines is the major route for their removal once released (11), then an inhibition of the reuptake process should make more catecholamine available to its receptor sites on the effector cell, resulting in an enhanced sensitivity. The fact that no change in sensitivity was observed, even though uptake was inhibited, may indicate that a certain level of reuptake inhibition must be achieved before sufficient extra transmitter is available to the receptor sites. The extra catecholamine made available by low levels of reuptake inhibition may be metabolised by the extra-cellular enzyme catechol-O-methyl transferase (COMT), or simply diffuse away, before it can bind to its receptor.

Failure to observe any change in myocardial sensitivity in this experiment, even though the reuptake of norepinephrine was inhibited, is not inconsistent with the in vitro studies. While (1–24)ACTH in vitro was found to both inhibit the reuptake of ^{3}H-norepinephrine (13) and to induce an enhanced myocardial sensitivity to norepinephrine (2) in a dose-dependent manner, the dose range was much broader with the inhibition of uptake. A reduction by a factor of 10 in the bath concentration of (1–24)ACTH producing a maximal effect still produced a significant reduction in ^{3}H-norepinephrine uptake, but completely abolished any enhanced myocardial sensitivity (2,13).

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