Altered hormone levels and circadian rhythm of activity in the WKY rat, a putative animal model of depression

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Although only single time point determinations have been made, WKY rats also appear to exhibit hormonal abnormalities of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid (HPA and HPT, respectively) axes, two axes that are often disrupted in depressed patients. For example, WKY rats show increased plasma ACTH after both chronic and acute stress relative to several other rat strains, including the Wistar rat (24, 56, 62), and have higher basal thyroid-stimulating hormone (TSH) and 3,5,3’-triiodothyronine (T3) levels relative to Wistar rats (63).

In human depression, the daily sleep/wake cycle and several hormonal rhythms, especially those of the HPA and HPT axes, are often disrupted (for reviews, see Refs. 20, 50, 69, 78). Due to these circadian rhythm alterations in depressed humans, we tested the hypothesis that the WKY rat would exhibit similar hormonal and activity rhythm alterations.

To test this hypothesis we examined the diurnal secretory patterns of ACTH, corticosterone, and TSH in freely moving WKY rats kept on a long-day (LD) cycle, relative to their outbred progenitor strain, the Wistar rat (45). We also measured plasma levels of T3 at a single time point in these two strains. In addition, we monitored activity rhythms in these strains under both LD and constant dark (DD) conditions. To investigate the intrinsic properties of the master circadian pacemaker, we measured the free running rhythm and the response of the activity rhythm after a light pulse while rats were under DD conditions.

MATERIALS AND METHODS

Experimental Protocol

Eleven male Wistar and eleven male WKY rats were obtained from Harlan Sprague Dawley at 10–11 wk of age. Immediately on arrival, rats were placed into individual cages in a light-tight room on a 14:10-h light-dark cycle (lights on at 0700, off at 2100 Central Standard Time). Animals were kept under constant ambient temperature (21 ± 1°C) with food and water available ad libitum. Rats were handled approximately four times a week to accustom them to the investigator in an effort to decrease stress during the 24-h period of blood sampling. Two weeks after arrival,

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animals were weighed and implanted with a jugular cannula to allow for serial blood sampling over a 24-h period. The 24-h bleed took place 2 days after the surgery.

At 6 mo of age, eight of the previously cannulated Wistar and nine WKY rats were placed into light-tight boxes to monitor daily activity rhythms. The light intensity in these boxes was ~300 lx (white light). Animals were maintained under a 14:10-light-dark cycle for a 3-wk period and were then transferred to DD for the remainder of the experiment. After 2 wk in DD, animals were exposed to a 15-min light pulse (white light) 1 h after activity onset. Exposure to light at this time is known to cause phase delays in the activity rhythm of rats (57). Activity rhythms were monitored in DD for a 2-wk period after the light pulse.

Two months after the 24-h bleed and before monitoring activity rhythms, an FST was administered to verify a depressive-like state in the WKY rat. Previous studies have shown that WKY rats exhibit high levels of immobility in the FST relative to several other rat strains, which has been interpreted as a high level of despair in this strain (e.g., Refs. 44, 56).

To obtain sufficient plasma volume for measuring T₃ levels, eight male Wistar and eight male WKY rats were killed by decapitation at 1700 in a separate study. Trunk blood was collected on ice in 15-ml conical tubes with EDTA (15.6 mg EDTA/tube). Plasma was collected and stored at −80°C. Levels of T₃ were determined by radioimmunoassay.

Jugular Cannulation and 24-h Blood Sampling

Before jugular cannulation, animals were anesthetized with an injection of ketamine (80 mg/kg ip) and xylazine (12 mg/kg ip). A small incision was then made in the neck to expose the jugular vein, and the short end of the cannula was inserted into the vein and securely tied off. The long end of the cannula was drawn out through a small incision in the back of the animal to enable serial sampling with minimal disturbance to the animal. Donor blood was prepared as previously described (76), with donor blood cells reconstituted in sterile saline to reach a hematocrit of 45–47%. Plasma was collected and stored at 80°C for subsequent determination of ACTH, corticosterone, and TSH hormone levels by RIA.

RIAs

All assays were done in duplicate. Corticosterone. The RIA was carried out as described previously (62). Briefly, 1–2 µl of plasma was incubated overnight with the primary corticosterone antibody raised against corticosterone 3-carboxymethyloxime-bovine serum albumin, with ¹²⁵I-labeled corticosterone conjugate as the tracer (ICN Pharmaceuticals, Costa Mesa, CA). The assay sensitivity was 16.7 pg/tube. The intra- and interassay coefficients of variation were 11.6 and 7.5%, respectively.

ACTH. As described previously (62), 25 µl of unextracted plasma was incubated for 2 days with a primary ACTH antibody that recognizes ACTH (1–24) and (1–39) on an equimolar basis (1:8,000 dilution, INCSTAR, Stillwater, MN) and incubated for 1 day with ¹²⁵I-labeled ACTH as the tracer (Amersham, Piscataway, NJ). The assay sensitivity was 1.5 pg/tube. The intra- and interassay coefficients of variation were 9.6 and 10%, respectively.

TSH. As described previously (64), 25 µl of unextracted plasma was incubated for 2 days with the primary TSH antibody (1:500,000 dilution; National Institute of Diabetes and Digestive and Kidney Diseases, Baltimore, MD), and incubated for 1 day with ¹²⁵I-labeled TSH tracer (rat TSH RP-2, iodinated by the Chloramine-T method). The assay sensitivity was 23.5 pg/tube (0.9 ng/ml). The intra- and interassay coefficients of variation were 8.1 and 6.7%, respectively.

T₃. T₃ RIAs were performed using ImmunoChem-coated tubes purchased from ICN Pharmaceuticals (Costa Mesa, CA), using 100 µl of unextracted serum and incubating with ¹²⁵I-labeled T₃ as tracer (provided) at 37°C for 1 h. The assay sensitivity was 24.7 ng/dl. The intra-assay coefficient of variation was 3.6%.

Activity Rhythm Data Collection and Analysis

An infrared sensor (Petite 101, Newark, NJ) was placed over each cage to monitor daily activity rhythms. The number of infrared beam crossings was continuously recorded and sent to an online data-acquisition system (Chronobiology Kit, Stanford Software Systems). Clocklab (Actimetrics, Evanston, IL), a rhythm analysis program, was used to analyze the activity rhythm data. While rats were kept on an LD cycle, the phase angle of entrainment was measured. In DD, the free-running period of the activity rhythm of the rats was measured, using the x² periodogram, 10 days before and 14 days after administration of the light pulse. The onset of activity was defined by the Clocklab program as follows: Clocklab searches for a best fit between the activity record and an activity pattern consisting of 6 h of inactivity followed by 6 h of activity. To find the most likely activity onset time, the program replaces each point in the activity record with “1” if it is in the top 80% of non-zero counts, or “−1” otherwise. The results are then convolved with a square wave that consists of −1 for the first 6 h and 1 for the next 6 h. The local maximum of this convolution within each 24-h period is taken as the activity onset time. To calculate phase shifts in the activity rhythm after the light pulse, Clocklab fit a line to the onsets of locomotor activity for 10 days before the light pulse and for 14 days after the light pulse. Then, a second line was retro-projected to the day of the light pulse, and the magnitude of the phase shift was calculated as the difference between the two lines.

FST

The FST procedure used was similar to the one described by Porsolt et al. (60). Briefly, animals were placed into a large
cylinder (30 cm × 45 cm) of 25°C water for a 15-min period. Twenty-four hours later, the rats were again placed into the cylinder of water for a 5-min period. All testing took place between 1100 and 1430. Activity during the posttest was video recorded for subsequent scoring. We used a time sampling technique for scoring, as previously described by Detke et al. (16), where time immobile, swimming, and climbing, measured every 5 s, were labeled as bins.

Statistical Analyses

A repeated measures one-way ANOVA was used to determine the statistical significance in hormone levels across the 24-h period between WKY and Wistar rats. Differences between the two strains at specific time points were also determined using Bonferroni’s post hoc test. A two-way ANOVA was used to measure statistical differences in free running period between Wistar and WKY rats both before and after the light pulse. A Newman-Keuls post hoc test was used to make pairwise comparisons where significant differences were seen. A one-way ANOVA was used to measure statistical differences in the magnitude of the phase shift between the two strains. A one-way ANOVA was also used to determine statistical significance in body weight (at the time of jugular cannulation) as well as immobility, climbing, and swimming behaviors in the FST between WKY and Wistar rats. Differences in plasma levels of T$_3$, taken from trunk blood, were also determined by a one-way ANOVA. Means are reported with standard error of measurement.

RESULTS

Confirming data from several other laboratories, including our own (i.e., Refs. 3, 39, 51), WKY rats exhibited increased immobility (WKY = 9.7 ± 1.3 bins vs. Wistar = 3.7 ± 0.87 bins, $F_{1,19} = 14.33, P < 0.01$) and decreased climbing (WKY = 5.8 ± 1.2 bins vs. Wistar = 11 ± 2.2 bins, $F_{1,19} = 5.86, P < 0.05$) in the FST, a behavioral test for despair. No differences were seen in time spent swimming between the two strains. WKY rats also weighed significantly less than Wistar rats (WKY = 397.5 ± 10.7 g, $F_{1,22} = 126.2, P < 0.0001$), similar to weights shown previously in these strains (55).

Diurnal Profile of Plasma ACTH and Corticosterone

Plasma levels of ACTH were similar between the two strains during the daylight hours. However, plasma levels of ACTH remained significantly higher for several hours after the peak in WKY rats relative to Wistar rats (see Fig. 1A). With the use of a one-way repeated measures ANOVA on the 24-h hormonal profile between the two strains, there were main effects of time ($F_{12,243} = 11.38, P < 0.001$) and strain ($F_{12,243} = 13.07, P < 0.01$), as well as a strain × time interaction ($F_{12,243} = 3.06, P < 0.001$). WKY rats exhibited significantly higher levels of ACTH relative to Wistar rats at 2200 and 0300 (WKY vs. Wistar).

Diurnal Profile of TSH and Single Time Point Levels of T$_3$

Levels of TSH were significantly higher in WKY rats relative to Wistar rats at several time points sampled during the 24-h period (see Fig. 2). With the use of a repeated measures ANOVA, there were main effects of time ($F_{12,211} = 8.45, P < 0.001$) and strain ($F_{12,211} = 46.17, P < 0.001$) and a strain × time interaction ($F_{12,211} = 3.69, P < 0.001$). WKY rats exhibited a significant peak of plasma TSH at 1100. There was a highly significant increase in levels of plasma TSH at 0800, 1100, 1400, 1700, and 0600 in WKY rats relative to Wistar rats ($P < 0.01$, Newman Keuls).

Plasma levels of T$_3$ in WKY rats, measured from trunk blood taken at 1700, were significantly higher relative to Wistar rats (WKY = 168.8 ± 6.5 vs. Wistar = 120.2 ± 9.8 ng/ml, $F_{1,14} = 17.16, P < 0.001$).
Activity Rhythms

While on an LD cycle, no differences were seen in the phase angle of entrainment between WKY and Wistar rats. However, while under DD conditions, WKY rats had a significantly shorter free running period relative to Wistar rats, and this difference was enhanced after the phase-delaying light pulse (see Figs. 3 and 4). With the use of a two-way ANOVA on free running period both before and after administration of the light pulse, there were significant main effects of strain ($F_{1,13} = 35.63, P < 0.001$), light pulse ($F_{1,13} = 63.95, P < 0.001$), and a strain $\times$ light pulse interaction ($F_{1,13} = 9.15, P < 0.01$). The initial free running period for the WKY rat was 24.0 h ($\pm 0.02$), significantly shorter than that of the Wistar rat, 24.1 h ($\pm 0.03$) ($P < 0.05$, Newman Keuls). After the phase-shifting light pulse, this difference in free running period increased as the free run-
ning period of WKY rats lengthened by only 9.6 min to 24.16 h (± 0.03), whereas the period of Wistar rats lengthened by 20.4 min to 24.44 h (± 0.04) (P < 0.01, Newman Keuls). WKY rats also exhibited a smaller phase delay in the activity rhythm after the light pulse: 40.2 (± 7.8) min phase delay in WKY rats as opposed to 83.4 (± 10.8) min delay in Wistar rats (F1,16 = 10.83, P < 0.01) (see Figs. 3 and 5).

DISCUSSION

We have demonstrated that WKY rats show prolonged plasma ACTH and corticosterone diurnal peaks, as well as hypersecretion of plasma TSH throughout the 24-h period and of plasma T3 sampled at a single afternoon time point relative to Wistar rats. In addition, WKY rats exhibit a shorter free running period, as well as smaller phase shifts and an attenuated lengthening of the free running period after a light pulse relative to Wistar rats while in DD.

Hypersecretions of ACTH and Corticosterone in WKY Rats

Both WKY and Wistar rats exhibited diurnal rhythms of plasma ACTH and corticosterone secretion similar to those described previously for the rat (e.g., Refs. 2, 4). In WKY rats, the peaks of both ACTH and corticosterone, which occur after lights off, were prolonged, as plasma levels remained high for at least 5 h in WKY rats while the diurnal peaks of ACTH and corticosterone began to decline immediately after lights off in Wistar rats.

The differences in plasma levels of ACTH and corticosterone between WKY and Wistar rats occurred during the active dark period following the diurnal peak. Depressed patients, particularly the melancholic subtypes, exhibit increased levels of plasma ACTH and cortisol across the 24-h period (17, 43). A closer examination of the plasma cortisol profile in these studies reveals that the greatest difference is seen after the diurnal peak, because the peak is prolonged in depressed patients relative to normal controls, similar to what we see in the WKY rat. A phase advance of the diurnal cortisol peak has also been seen in depressed patients (43), which was not mirrored in the WKY rat.

The increased and prolonged peaks of ACTH and corticosterone in the WKY rat are more likely due to defective glucocorticoid negative feedback (79) than to increased corticotropin-releasing hormone (CRH) at this time (12, 49), as CRH mRNA binding and peptide content measured at single time points are not different in WKY rats relative to several other rat strains (24, 41, 62). However, although WKY rats seem to respond normally to dexamethasone treatment (23) and have hippocampal glucocorticoid or mineralocorticoid receptor binding and mRNA levels (24) similar to several other rat strains, adrenalectomy and corticosterone replacement have little or no effect on several glucocorticoid-dependent measures (62), including the dramatically elevated pituitary pro-opiomelanocortin mRNA in WKY relative to Wistar rats (61, 62). These data suggest that WKY rats may have a decreased sensitivity to glucocorticoids, which may be responsible, in part, for the prolonged diurnal peak of ACTH.

Hypersecretion of TSH and T3 in WKY Rats

WKY rats, whose plasma TSH levels were much higher throughout the 24-h period relative to Wistar rats, exhibited a clear diurnal peak of plasma TSH, similar to that reported in other rat strains (e.g., Refs. 34, 65, 84). The finding that Wistar rats showed no discernible diurnal rhythm could be due to their low-to-normal TSH levels, which were close to the assay sensitivity. Interestingly, the elevated plasma TSH levels in WKY rats were not caused by a relative hypothyroidism, as T3 levels were also slightly elevated in this strain.

Depressed humans exhibit alterations of the HPT axis, including both subclinical hyper- and hypothy-
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Altered Activity Rhythms Under DD Conditions in WKY Rats

No differences were noted in the activity profile of WKY relative to Wistar rats while under LD conditions. However, WKY rats did exhibit activity rhythm differences while in DD relative to Wistar rats. WKY rats exhibited a free running period of ~24 h, similar to what has been found previously in this strain (57, 66). The Wistar rat, on the other hand, had a free running period of 24.1 h, similar to previous findings in both Wistar and Sprague-Dawley rats (e.g., Refs. 10, 28). This difference suggests that the master pacemaker in WKY rats is running more quickly than that of the Wistar. In addition, the Flinders Sensitive Line, another genetic rat model of depression, has shown a similar difference in free running period relative to its control, the Flinders Resistant Line (71), suggesting a short free running period may be a common phenotype in animal models of depression. The difference in free running period between the two strains was enhanced after administration of a light pulse, inasmuch as the free running period in WKY rats increased by only 10 min, whereas the free running period of Wistar rats increased by ≥20 min. In addition, the phase delay of the activity rhythm in response to a light pulse was significantly smaller in WKY rats relative to Wistar rats. These differences suggest that the circadian pacemaker of WKY rats may be less responsive to light, thereby causing possible alterations in daily rhythm patterns of this strain.

It is difficult to measure activity rhythms in depressed patients, as these studies require temporal isolation to exclude environmental influences on the properties of the circadian clock. However, although controversial, there are some data demonstrating that patients with depression may have an altered sensitivity to light (see Refs. 20, 37).

Previous studies have found that spontaneously hypertensive rats (SHR) have a similar or shorter free running period than the normotensive WKY rats (57, 66, 68). Interestingly, when the hypertensive and hyperactive traits are separated by the selective breeding of progeny from a WKY and SHR cross (26), the hypertensive substrain exhibits a shorter free running period than the hyperactive substrain (67). These data provide an indirect link between free running period and hypertension and may offer a possible explanation for the short free running period in SHR. In addition, given that SHR and WKY rats are genetically related (45), it is not surprising that the free running period of both WKY rats and SHR is shorter than other rat strains.

The physiological basis for the smaller phase delays of the activity rhythm, as well as a decreased lengthening of the free running period after a light pulse in WKY rats relative to Wistar rats, is unknown. Rosennasser and Plante (68) found that with increased light intensity, the free running period of WKY rats increased significantly more than that of the SHR, and thus have suggested that WKY rats have an increased sensitivity to light compared with SHR. In addition, an increase in light intensity results in a decreased lengthening of the free running period in SHR relative to WKY rats (66). However, in the present study, the WKY rat exhibited a decreased responsiveness to the phase-shifting light pulse compared with Wistar rats, suggesting that both SHR and WKY rats may have a decreased sensitivity to light. Although not confirmed, the decreased response of the WKY rat to a phase-shifting light pulse may be due to a difference in the shape of the phase response curve, because the delay/advance region of the phase response curve is related to the length of the free running period (15). The attenuated lengthening of the free running period in WKY relative to Wistar rats may be due to the observed smaller phase delays of the activity rhythm of this strain. In support of this hypothesis, light pulses resulting in large phase delays of the activity rhythm consistently lead to a lengthening of the free running period in several species, including the rat, whereas small phase delays do not consistently result in period lengthening (59, 74).

Activity Rhythm and Hormonal Alterations in the WKY Rat are Similar to Other Animal Models of Depression

HPA dysregulation has been demonstrated in several animal models of depression. For example, rats...
exposed to chronic mild stress exhibit several depressive-like behaviors (83) and have increased levels of basal corticosterone 24 h after termination of the stress procedure relative to levels before chronic mild stress exposure (5). In addition, the olfactory bulbectomy model of depression exhibits increased plasma levels of corticosterone in the early morning hours, right after lights turn on (46). Prenatal stress, another animal model of depression, results in a phase advance of the corticosterone rhythm (35). To our knowledge, thyroid function has not been assessed in other animal models of depression.

The depressive-like behavior in the FST has been shown to be dependent on both glucocorticoids and thyroid hormones (30, 31). Removal of glucocorticoids by adrenalectomy in the rat decreases immobility in the FST, which is reversed by treatment with glucocorticoids (29). In contrast, thyroidectomy and a state of mild hypothyroidism increase immobility in the FST, which is reversed by L-thyroxine treatment (38).

A shorter free running period can also be observed in other animal models of depression. For example, the Flinders Sensitive Line, a genetic line bred for anticholinesterase sensitivity, has a free running period of ~24 h, similar to our results in the WKY rat, significantly shorter than the control Flinders Resistant Line (71). In addition, rats exposed to prenatal stress exhibit depressive-like behavior in adulthood and also have a shorter free running period relative to control rats not exposed to prenatal stress (81). On the other hand, chronic or severe stress-induced depression models, in which only short-term behavioral changes are seen (i.e., chronic mild stress, defeat stress, learned helplessness), result in no change or in a lengthening of the free running period (i.e., Refs. 48, 72, 73), suggesting that a shortened free running period may be associated with long-term endogenous depressive-like behavior in rodents.

In conclusion, the present study is the first to demonstrate prolonged ACTH and corticosterone peaks, as well as both hypersecretion of TSH and T3, in WKY relative to Wistar rats. In addition, WKY rats exhibited a shorter free running activity rhythm, an attenuated lengthening of the free running rhythm, and smaller phase shifts of the activity rhythm after a light pulse relative to Wistar rats. The sustained peak of ACTH in the presence of high levels of glucocorticoids and the increased levels of TSH in the presence of elevated levels of T3 suggest that the glucocorticoid and thyroid negative feedback systems are not functioning properly in WKY rats. Smaller phase shifts of the activity rhythm and an attenuated lengthening of the free running period after a light pulse in WKY rats suggest that these animals may be less sensitive to the effects of light. Future work in this animal model will determine whether the hormonal or activity rhythm abnormalities are causally related to the well-established depressive-like behavior of the WKY rat. In addition, this strain can be used to elucidate the connection between HPA and HPT dysregulation. Inasmuch as many of the hormonal and activity rhythm characteristics found in the WKY rat are homologous to those found in other animal models of depression, as well as in depressed humans, our data provide further support for the WKY rat as a valid animal model of depression.

Perspectives

Although no animal model can represent an exact replica of human affective disorders, the biological alterations we describe here, together with the well-established behavioral deficits found in the WKY rat, provide strong evidence that this strain is a valid animal model of depression. Furthermore, because the WKY is an inbred strain, it may be particularly useful for determining if shared genetic elements underlie the behavioral, hormonal, and rhythm phenotypes seen in the WKY rat. Although premature, the discovery of the genetic bases for these abnormalities in the WKY rat may provide preliminary information regarding possible genetic abnormalities in human depression.

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