Kinetics and Persistence of Cardiovascular and Locomotor Effects of Immobilization Stress and Influence of ACTH Treatment

Esther L. Sabban^a Nina Schilt^a Lidia I. Serova^a Shreeharsha N. Masineni^b
Charles T. Stier, Jr.^b

Departments of ^aBiochemistry and Molecular Biology and ^bPharmacology, New York Medical College, Valhalla, N.Y., USA

Key Words

Adrenocorticotrophic hormone • Blood pressure • Heart rate • Locomotion • Radiotelemetry • Stress

Abstract

Stress triggers crucial responses, including elevated blood pressure and heart rate (HR), to handle the emergency and restore homeostasis. However, continuation of these effects following cessation of the stress is implicated with many stress-related disorders. Here, we examine the kinetics and persistence of cardiovascular and locomotor responses to single and repeated immobilization stress (IMO), with and without prior treatment with adrenocorticotrophic hormone (ACTH). Radiotelemetry probes were implanted into male Sprague-Dawley rats to continually monitor mean arterial pressure (MAP), HR and locomotor activity. Rats were subjected to IMO for 2 h daily (10 a.m. to noon, 6 consecutive days). The first IMO induced the greatest change in MAP (about 30 mm Hg) and HR (about 200 bpm). Following each IMO, MAP and HR were elevated during the remaining light phase and in the subsequent dark phase, HR was lower than prior to IMO. We further examined whether elevation of ACTH to a level similar to IMO will elicit similar effects, and if it will alter subsequent responses to IMO. Injection of ACTH

(13 IU/kg, s.c.) triggered a short-lived rise in MAP, and decreased HR. After six daily injections of ACTH and recovery time (8 days), rats were immobilized as above. The cardiovascular responses were similar during the IMO, but the ACTH-pretreated group displayed differences following cessation of the IMO. In addition, IMO led to a large reduction of locomotor activity during the dark (normally active) phase to levels similar to the light phase. Following the IMOs, locomotor activity recovered more slowly in the ACTH-pretreated group. The study revealed that IMO-triggered cardiovascular and locomotor responses are evident after termination of the stress. In addition, prior exposure to ACTH delayed recovery in cardiovascular and locomotor functions following cessation of stress.

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Introduction

Acute stress triggers important neuroendocrine responses that enable the organism to restore homeostasis and survive. However, when stress is excessive or repeated over a long period of time, the response is not only adaptive, but also maladaptive [1]. Stress plays a major role in the increased incidence of a number of common

life-threatening disorders [reviewed in 2, 3]. Prominent among these are cardiovascular disorders including myocardial infarction and hypertension [reviewed in 4].

Key components mediating the broad physiological range of responses to stress include the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, sympathetic nervous system and adrenomedullary catecholaminergic system leading to release of adrenocorticotrophic hormone (ACTH), glucocorticoids, catecholamines and costored neuropeptides into the circulation [5–8]. However, the individual contributions of these factors to the stress response are still unclear and difficult to discern, as activation of the HPA axis and stimulation of catecholaminergic systems are interrelated at many levels.

Several patterns of ACTH responses have been identified depending on the stress paradigm and influenced by differential regulation of the hypothalamic regulators corticotropin-releasing hormone and vasopressin [9]. In order to dissociate the effects elicited by activation of the HPA axis from other aspects of stress (such as the adrenomedullary response), bolus injections of ACTH have been used. Repeated daily injections of ACTH elicited elevated mRNA for catecholamine biosynthetic enzymes, tyrosine hydroxylase and dopamine β -hydroxylase in rat superior cervical ganglia that were as pronounced as observed with immobilization stress (IMO) [10]. Exposure of these rats to IMO did not induce further changes. Moreover, the mRNA for the melanocortin 2 receptor, the ACTH receptor well characterized in the adrenal cortex, was found to be expressed in sympathetic ganglia, both superior cervical ganglia and stellate ganglia, and elevated in rats exposed to IMO [11]. This suggested that the stress-triggered rise in ACTH can play a crucial role in regulation of norepinephrine (NE) biosynthesis in sympathetic ganglia [10].

ACTH, which belongs to a family of peptides that are derived from proopiomelanocortin, has been reported to have a variety of cardiovascular effects in various species depending on its site of action [12–15]. In this regard, about 80% of patients with Cushing's syndrome with ectopic ACTH secretion display elevated blood pressure (BP), which is suggested to result from the abnormally high glucocorticoids [16]. While ACTH triggers elevated adrenal glucocorticoids, the high levels of plasma ACTH following adrenalectomy (a situation where circulating adrenal hormones including corticosterone and epinephrine are nearly absent) are associated with overactivation of the sympathetic nervous system and increased circulating NE levels [17]. These results suggest that repeated exposure to ACTH may lead to hyperactivity of the NE

sympathetic system. This led us to hypothesize that ACTH administration may have long-term effects on the cardiovascular response to stress.

Radiotelemetry has been useful to monitor changes in BP, heart rate (HR) and behavior response in freely moving animals with many models of stressors, such as restraint, handling, air jet stress, chronic mild stress, shaker stress, exposure to novelty and others [18–23]. To our knowledge, telemetry has not been previously used to assess the response to IMO. An early study revealed that repeated daily IMO over several weeks elevated BP [24]. In that study, daily IMO elicited mildly elevated BP, as measured by tail-cuff plethysmography, at 2 weeks, the shortest time examined, and pronounced elevations after 4 weeks. The BP declined gradually during the 2 weeks following the IMOs.

In the present study, we examined the kinetics and persistence of cardiovascular and locomotor responses to IMO in normotensive rats with and without ACTH pretreatment in animals instrumented with radiotelemetric probes for chronically monitoring mean arterial pressure (MAP), HR and locomotor activity. We tested the hypothesis that exposure to the strong stressor of immobilization, and perhaps even ACTH treatment alone, would evoke long-lasting changes in MAP, HR and in locomotor activity. We also tested the hypothesis that the responses to IMO would be altered by prior exposure to ACTH.

Materials and Methods

Experimental Animals

All experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23) and approved by the Institutional Animal Care and Use Committee. Pathogen-free adult male Sprague-Dawley rats (275–300 g) were purchased from Taconic Farms (Germantown, N.Y., USA). Animals were housed 2 per cage, one experimental and one companion, in order to avoid isolation stress [25]. They were housed in a room maintained at an ambient temperature of $23 \pm 2^\circ\text{C}$ with a 12-hour light (6 a.m. to 6 p.m.) and 12-hour dark (6 p.m. to 6 a.m.) cycle and were given a standard rodent diet (Purina Lab Chow No. 5001; Ralston-Purina, St. Louis, Mo., USA) and allowed water ad libitum.

Radiotelemetry

Radiotelemetry probes (TA11PA-C40; Data Sciences International, St. Paul, Minn., USA) were implanted using sterile techniques in rats anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Abbott Laboratories, North Chicago, Ill., USA) as previously described [26–28]. Briefly, a midline abdominal incision was made and the telemetry probe catheter was introduced retrogradely into the descending aorta through a puncture site which was then sealed with tissue adhesive (3M Vetbond 1469; 3M Ani-

Fig. 1. Experimental procedures.

mal Care Products, St. Paul, Minn., USA). The body of the telemetry probe was then sutured to the abdominal wall upon closing the midline incision. Animals were allowed 10 days to recover from surgery and to acclimate to the experimental environment.

The HR, systolic, diastolic BP, MAP and locomotor activity were sampled over a 10-second period at 5-min intervals for the duration of the study. The locomotor activity of the animals was detected as a consequence of changes in received signal strength from the transmitter which occur upon movement of the animals. Transmitted data were harvested and analyzed using the Dataquest Art Silver Data-Acquisition System (Version 1.10, Data Sciences International, St. Paul, Minn., USA).

Procedures

Figure 1 shows a diagram of the experimental procedures.

Animals were injected with ACTH under conditions which were previously found to elevate expression of NE biosynthetic enzymes in sympathetic ganglia [10, 29]. Rats were given ACTH injections once daily (at 10 a.m.) for 6 consecutive days. ACTH 1–39 (catalog No. A6303; Sigma Aldrich, St. Louis, Mo., USA) was freshly dissolved in vehicle (sterile saline, 0.9% NaCl) at a concentration of 13 IU/ml and injected subcutaneously at a volume of 1 ml/kg to provide a dose of $13 \text{ IU} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (1 mg = 80 IU). Rats were then allowed to rest for 8 days. This time was chosen to enable the anticipated elevation in tyrosine hydroxylase and dopamine β -hydroxylase expression in the sympathetic ganglia [10, 29] to be manifested in increased NE biosynthetic capacity in sympathetic nerve endings.

Then, animals, with and without prior ACTH injections, were subjected to repeated IMO. Controls were untreated or injected with vehicle. Animals were immobilized on metal platforms for 2 h once daily for 6 consecutive days exactly as in our previous studies [e.g. 30–33]. The IMO was performed between 10 a.m. and noon [6]. The head was placed within a loop to minimize movement and the forelimbs and hindlimbs secured with surgical tape to metal mounds attached to the platform. After the IMO the rats continued to be monitored by telemetry for at least an additional 4 days.

Determination of Plasma ACTH and Corticosterone Levels

Plasma ACTH and corticosterone concentration was determined in parallel experiments performed in separate groups of

6–8 rats that were exposed to the identical experimental treatments as the rats with telemetry probes implanted. One group of rats underwent a single session of IMO, and a second group was injected with ACTH. At various times the rats were killed by rapid decapitation, as plasma ACTH can increase markedly with anesthesia [34]. Trunk blood was collected into chilled EDTA tubes.

ACTH levels were determined by using the RSL ^{125}I hACTH kit (ICN, Costa Mesa, Calif., USA) according to the manufacturer's protocol. ACTH concentration was analyzed in 0.1 ml of plasma, which was incubated with antiserum to ACTH and hACTH- ^{125}I in polystyrene tubes for 20 h at 4°C. After centrifugation, precipitates were counted in a gamma counter. The standard curve was obtained from standards of ACTH in the range from 10 to 1,000 pg/ml and used for quantification. The intra- and interassay coefficients of variation for the ACTH assay were 8.7 and 11.8%, respectively.

For corticosterone measurement, a corticosterone ^{125}I RIA kit (MP Biomedicals, LLC, Orangeburg, N.Y., USA) was used. Plasma was diluted 1:200 and incubated with ^{125}I -labeled corticosterone and corticosterone antiserum at room temperature for 2 h. After centrifugation, precipitate radioactivity was measured in a gamma counter. Samples were quantified against a standard curve constructed from serial dilutions of corticosterone from 1,000 to 25 ng/ml. The intra- and interassay coefficients of variation were 5.0 and 8.2%, respectively.

Statistical Analysis

All radiotelemetry data are reported as mean $\pm$ SE calculated for 5 min or averaged over greater intervals as indicated in the figure legends. The data from the two control groups (with no pretreatment and pretreated with saline injections) were combined as they were similar. Data with only one grouping variable were analyzed statistically by one-way analysis of variance (ANOVA) with repeated measures followed by post-hoc analysis using the Bonferroni comparison of means or t tests as appropriate. Data with two grouping variables (ACTH pretreatment and time) were analyzed by two-way ANOVA for main effects and interactions. The version 4.01 of the GraphPad Prism and InStat statistical software packages (GraphPad Software, San Diego, Calif., USA) was used for data analysis. In all cases, $p < 0.05$ was considered statistically significant.

Fig. 2. Plasma ACTH (a) and corticosterone (b) levels with injection of ACTH or IMO. In animals (6–8/group) not implanted with telemetric probes, plasma ACTH levels were measured with the indicated times of IMO or after injection of vehicle or ACTH (13 IU/kg). Data points are expressed as mean $\pm$ SE. * $p < 0.05$ vs. time 0; $^{\dagger} p < 0.05$ vs. the vehicle-treated group at the same time point; $^{\#} p < 0.05$ ACTH-treated vs. IMO.

Results

Acute MAP and HR Responses to IMO, Injection of ACTH or Vehicle

We administered ACTH (13 IU/kg, s.c.) under conditions which were previously found to elevate gene expression of catecholamine biosynthetic enzymes in rat sympathetic ganglia [10, 29]. First, we examined the plasma ACTH and corticosterone attained with these injections compared to levels during IMO (fig. 2). Groups of Sprague-Dawley rats, without telemetric probes implanted, were either injected with saline, ACTH or subjected to IMO. Changes in plasma ACTH are shown in figure 2a. Plasma ACTH levels were 40.0 ± 3.4 pg/ml in animals that received no treatment. By 15 min of IMO or after injection of ACTH, plasma ACTH was increased to comparable levels which were $1,100 \pm 130$ and $1,300 \pm 100$ pg/ml, respectively. However the rise in ACTH was shorter-lived with the ACTH injection as compared with IMO. Plasma corticosterone levels 15 min after injection of ACTH were also similar to those attained with 15 min of IMO (fig. 2b). However, while corticosterone was further elevated after 60 min of IMO and remained high throughout the 2 h of IMO, by 60 min after the ACTH injection, corticosterone levels did not differ from those in the vehicle-injected animals.

Next, we determined the cardiovascular effects of ACTH injections or IMO individually on MAP and HR. For these experiments, radiotelemetric probes were implanted and MAP and HR monitored at 5-min intervals during and after IMO or injections on the first day of

treatment. ACTH and IMO both triggered a rapid rise in MAP of approximately 30 mm Hg (fig. 3a). MAP remained markedly elevated throughout the 2-hour period of IMO and gradually decreased after its cessation at 120 min. Although MAP with ACTH injection reached levels that were comparable to those at the beginning of IMO, MAP declined and returned to baseline by 45 min after injection. The isovolumic injection of the vehicle for ACTH (0.9% NaCl) was associated with an increase in MAP only within the first 10 min. IMO also triggered a rapid increase in HR of almost 200 bpm, which like MAP was highly sustained throughout the 2-hour period of IMO and gradually declined thereafter (fig. 3b). Unlike IMO, ACTH or vehicle injection only transiently increased HR, which was elevated approximately 50 bpm after 5 min. Thereafter, HR markedly decreased to levels below baseline in animals injected with ACTH while HR in animals injected with vehicle slowly returned to pre-treatment levels. HR was indistinguishable from baseline by 30 min after injection of ACTH or vehicle.

The MAP (fig. 4a) and HR (fig. 4b) responses to repeated daily injections of ACTH, compared to vehicle, are shown at 15 min after the injections. As seen in figure 3, at this time the response to vehicle had subsided and the ACTH response was still maximal. The elevation in MAP and decline in HR with ACTH were greatest on the initial day of injection. MAP was significantly elevated on days 1, 2 and 5 and HR was significantly reduced on days 1–4 of ACTH injection compared to the vehicle.

Fig. 3. Effect of initial ACTH injection or IMO on MAP and HR. The MAP (**a**) and HR (**b**) were measured by radiotelemetry before, during, and after a 2-hour period of IMO ($n = 8$) or the injection of 13 IU/kg ACTH ($n = 9$) or vehicle ($n = 5$). The data are shown as means $\pm$ SE taken at 5-min intervals.

Fig. 4. Effect of repeated daily ACTH injections on MAP and HR. The MAP (**a**) and HR (**b**) expressed as a ratio of the response to ACTH (13 IU/kg) relative to vehicle at 15 min after the injections is shown for each of 5 consecutive days. Data are reported as the mean $\pm$ SE. * $p < 0.05$ compared with the value on day 1; [†] $p < 0.05$ compared with the response to vehicle on the same day.

Fig. 5. Diurnal variations in MAP and HR prior, during and after the 6 days of IMO. Rats, with and without prior administration of 6 daily ACTH injections, were monitored for MAP (**a**) and HR (**b**) for 2 days before, during 6 days (D1–D6) of IMO from 10 a.m. to noon, and for 4 days afterwards. Data are mean $\pm$ SE every 2 h

during the light (L) and dark (D, shaded) phases. The beginning of each IMO is shown by an arrow. The dashed line shows the nadir of MAP and HR prior or subsequent to the IMO. * $p < 0.001$ for peak values on D1 IMO vs. D2–D6 for controls; † $p < 0.001$ for peak values on D1 IMO vs. D2–D6 for ACTH pretreated.

Fig. 6. The MAP and HR in light and dark phases subsequent to IMO. Rats were monitored for MAP and HR during the remainder of the light phase (noon to 6 p.m.) (**a**, **c**) after the 2-hour period of IMO and during the subsequent dark phases (6 p.m. to 6 a.m.) (**b**, **d**) on each of 6 consecutive days as well as 1 day before

(-1) and 4 days after (1+–4+) the IMO. Data from animals, with ($n = 8$) and without ($n = 9$) prior exposure to ACTH, are presented as the mean $\pm$ SE. * $p < 0.05$ vs. day -1 for control group; † $p < 0.05$ vs. day -1 for ACTH-pretreated group; # $p < 0.05$, ACTH-pretreated vs. control.

MAP and HR Responses to Repeated IMO with and without Prior ACTH Treatment

The changes in MAP and HR in response to daily 2-hour exposures to IMO were followed in control rats and in rats which had previously received daily ACTH injections for 6 consecutive days. The diurnal variations in MAP (fig. 5a) and HR (fig. 5b) before, during and after the 6 daily 2-hour intervals of IMO (10 a.m. to noon) are shown. The initial MAP and HR of both groups were similar prior to the IMO while the peak elevations were significantly higher in both groups with the first IMO. The peak responses in MAP and HR to IMO were similar with and without prior exposure to ACTH. HR tended to recover more slowly in the ACTH-pretreated group on the first few days of IMO. During the 6 days of IMO, neither the MAP nor HR fell back to the nadir observed before or after the 6 days of stress.

To examine more closely the effects of IMO each day after its cessation, the average MAP and HR were calculated for the remainder of the light phase (noon to 6 p.m.) and for the following dark phase (6 p.m. to 6 a.m.) (fig. 6). In the remaining light phase (fig. 6a), two-way ANOVA revealed that MAP depended significantly on time ($F_{10,153} = 53.8$, $p < 0.0001$) and ACTH pretreatment ($F_{1,153} = 5.8$, $p < 0.05$). There was an interaction between ACTH pretreatment and time ($F_{10,153} = 5.2$, $p < 0.01$). The average MAP remained significantly elevated after IMO in rats in the control group on days 1 to 6 and in the ACTH-pretreated group on days 1 and 6. On the day following the last IMO (day 1+), average MAP approached levels observed on day -1. In the dark phase (fig. 6b), the initial average MAP was higher than in the light phase and did not change during the course of the experiment. Nevertheless, there was a slight, but significant, elevation

Fig. 7. Diurnal changes in locomotor activity prior to, during and following ACTH injections or IMO. Average locomotion during the light (**a**) and dark (**b**) phases excluding the 2-hour period of IMO on each of 6 consecutive days as well as 1 day before (–1) and 4 days after (1+–4+) the IMO series in rats with ($n = 8$) and without prior ACTH pretreatment ($n = 9$). They are compared to locomotor activity of rats not immobilized but injected for 6 days with ACTH ($n = 8$). Data are presented as the mean $\pm$ SE. * $p < 0.05$ vs. day –1 for control group; † $p < 0.05$ vs. day –1 for ACTH-pretreated group; # $p < 0.05$, ACTH-pretreated vs. control; § $p < 0.05$ vs. day –1 for not immobilized group.

compared to pre-IMO (day –1) only in the control group on day 2+.

Like MAP, during the light phase the increases in HR were very pronounced (fig. 6c). Two-way ANOVA revealed that HR in the light phase significantly depended on time ($F_{10,153} = 17.1$, $p < 0.0001$) and ACTH pretreatment ($F_{1,153} = 8.9$, $p < 0.0001$). The average elevation in HR following the first IMO was 80 and 110 bpm in the control and ACTH-pretreated groups, respectively. The average HR was elevated on each of the subsequent days of IMO, with the most pronounced effect on day 1. The HR returned to control (day –1) levels upon cessation of IMO treatments (day 1+).

During the dark period (fig. 6d), HR was affected by time ($F_{1,157} = 14.7$, $p < 0.0001$) and ACTH pretreatment ($F_{1,153} = 11.4$, $p < 0.001$) with a significant interaction ($F_{10,153} = 14.4$, $p < 0.0001$). The HR was reduced (20–30 bpm) on each night after IMO in the control group and on days 4 and 6 in the ACTH-pretreated group. Interestingly, animals that were pretreated with ACTH differed from the control group on day 1 and day 2 and did not show a reduction in HR during the dark phase response. The ACTH group also showed a tendency toward a greater elevation ($p < 0.06$) in HR in the light phase on these days.

Locomotor Activity

The diurnal variations in locomotor activity are shown in figure 7. Injections of ACTH alone had no effect on the diurnal variation in locomotor activity which was high during the dark (active) phase and low during the light (inactive) phase. However, during the 6 days of IMO, the locomotor activity in the dark phase was dramatically reduced to levels that were comparable to those observed during the light phase in both groups of rats, with and without ACTH pretreatment. Upon cessation of the stress on day 6, locomotor activity during the dark phase remained significantly lower 4 days afterwards. It only gradually returned towards control levels in both groups. Two-way ANOVA revealed that in the dark phase there were significant main effects of time and ACTH pretreatment on locomotor activity ($F_{1,153} = 65.41$, $p < 0.0001$; $F_{1,153} = 51.3$, $p < 0.0001$, respectively), with a significant interaction ($F_{10,153} = 4.5$, $p < 0.0001$). In the ACTH-pretreated group, there was diminished recovery of locomotor activity which was significantly different from the control group 2–4 days after termination of the immobilizations.

Discussion

The present study examined the kinetics and persistence of changes in cardiovascular and locomotor functions in response to IMO in rats with or without prior exposure to ACTH. The results show the strong stressor of immobilization triggers cardiovascular and locomotor responses which are evident not only during the application of the stress, but for many hours thereafter. The cardiovascular responses to IMO, or to ACTH injection, are most robust for the first compared to subsequent repeated exposures. Even a single exposure to the strong stress of IMO leads to complete loss of the diurnal variation in

spontaneous locomotor activity. In addition, pretreatment with ACTH modulates several of these responses, not evident during, but rather after exposure to the stressor. These findings are important not only for the usefulness of the immobilization model but also for an understanding of the lingering influences of exposure to stress.

As expected [19, 35], the initial exposure to IMO or ACTH elicits the most robust increase in BP and HR. Interestingly, the findings also revealed that even a single exposure to IMO was sufficient to prevent the rise in spontaneous locomotor activity during the dark (normally active) phase. Decreased physical activity during the dark phase was previously observed with mice subjected to a model of psychosocial stress that involves introducing an intruder into the cage of a resident male animal. The subordinate animals, but not the dominant ones, displayed reduced physical or locomotor activity during the normally active dark phase [36]. Chronic stress has been shown to produce anxiety and depression in animal studies [37–39]. Reduced locomotor activity is characteristic of the chronic mild stress (CMS) model of depression. However, in the CMS model, locomotor activity diminished gradually over a period of 4 weeks [21], while in the present study the normal increase in dark phase activity was completely lost already on the night following the first IMO. Upon cessation of the IMO sessions the reduction in locomotor activity was maintained up to 4 days, the longest time examined – a relatively long time in the life span of a rat, which is about one fortieth the human life span. In the CMS model of depression, the decline in spontaneous locomotor activity returned to near basal levels relatively rapidly after termination of the stress [21].

A novel aspect of the study is the influence of prior treatment with ACTH on the subsequent response to stress. Acute ACTH produced pressor and bradycardic responses relative to vehicle injection. The finding that injection of ACTH elevates MAP and glucocorticoids (after 15 min) as high as attained with IMO suggests that ACTH or glucocorticoids may contribute to the rapid elevation in BP seen soon after commencing this stress. Further studies using ACTH and glucocorticoid receptor antagonists will be needed to evaluate their relative contribution. However, the responses to ACTH were not sustained, compared to IMO, and clearly, other factors come into play. With ACTH injections, HR unlike BP, did not increase compared to the vehicle, but instead declined and this probably reflects a baroreflex adjustment in response to the rapid rise in BP in the absence of any direct

cardioaccelerator effect of ACTH. This is in contrast to the marked and sustained increase in HR throughout the 2-hour IMO period, which probably reflects an increase in sympathetic drive and/or withdrawal of parasympathetic output to the heart.

There are conflicting results in the literature regarding the effects of ACTH on BP. Acute intravenous injections with a low dose of ACTH fragment (1–24) have been reported to have a transient (less than 2 min) depressor effect combined with a brief tachycardia [40]. However, repeated (twice daily) administration of depot ACTH (1–24) produced sustained BP elevation in normotensive and hypertensive individuals as well as in experimental animals [41]. In the present study, using once daily injections of ACTH (1–39) had no effect on baseline BP, but we did not go beyond 6 injections. The differences from the prior study, which found hypertension established after 4 days, may be due to the formulation of the ACTH (saline dissolved vs. depot preparation) or dosing schedule (once vs. twice daily).

One of the main findings of the present study is that ACTH elicited long-term effects on the kinetics of recovery of cardiovascular and locomotor functions after stress, despite discontinuation of its administration. The ACTH-pretreated animals displayed a slower recovery of locomotor activity than the controls. Moreover, there was a significant influence of ACTH pretreatment on HR (light and dark phases) and MAP (light phase). This was especially pronounced during the first few days of IMO. The delayed recovery of HR after cessation of IMO in the ACTH-pretreated group may have clinical implications. Indeed, a delayed recovery in HR after submaximal exercise training in individuals with no evidence of cardiovascular disease is reported to be a strong predictor of early mortality [42, 43]. Studies comparing the response to stress in normotensive male rats, with and without genetic risk for hypertension, suggest that the rate of recovery of cardiovascular changes following stress may be among the earliest precursors to the development of hypertension [44]. Thus, the greater elevation in HR after cessation of IMO, observed here in ACTH pretreated animals, suggests that chronic elevations of ACTH may condition to poor cardiovascular outcome.

What is the mechanism for the persistent effects of ACTH? Classically, the release of pituitary ACTH under direction of corticotropin-releasing hormone from the hypothalamus resulting in synthesis and release of glucocorticoids from the adrenal cortex and the subsequent feedback inhibition is a well-characterized response to stress [reviewed in 45]. Injections of ACTH led to parallel

elevation of corticosterone during the first 15 min although after that its level dramatically declined. Thus, the most parsimonious explanation for the effects of ACTH on stress response and especially recovery is the consequence of release of corticosterone, modulating cardiovascular tone since ACTH was administered into adrenal intact animals. Glucocorticoids play an important role in arterial pressure homeostasis, and glucocorticoids are implicated in the pathogenesis of human hypertension [reviewed by 46–48]. Corticosterone has been shown to be involved in the cardiovascular response of rats to mild psychological stress, such as open field novelty, with an important role attributed to putative brain mineralocorticoid receptors, while additional acute or chronic occupation of glucocorticoid receptors had differential and sometimes opposing effects [49].

The effect of ACTH treatment on expression of catecholamine biosynthetic enzymes in sympathetic ganglia should also be considered as a contributing factor. The role of the sympathetic/parasympathetic balance was shown to be important in stress-induced changes in HR, BP and indices of variance [23]. Whether or not this is a direct effect of ACTH remains to be determined in light of the finding of MC2R mRNA expression in rat superior cervical and stellate ganglia. Further experiments with adrenalectomized animals will be needed to clarify this point. However, the situation could be complex as adre-

nalectomy which also elevates circulating ACTH has been found to increase HR, but not BP, in Sprague-Dawley rats [49]. It is attractive to postulate that the pretreatment with ACTH leads to greater sympathetic activation due to increased NE biosynthesis. This possibility is supported by our findings that injection of ACTH can increase the expression of catecholamine biosynthetic enzymes in sympathetic ganglia of adrenalectomized rats [50].

In summary, the present findings indicate the importance not only to the response during the stress, but also following cessation of exposure to a strong stressor. Following the cessation of IMO, BP and HR remained above control values in the light phase and did not reach the pre-IMO values prior to exposure to the stress. The diurnal variations in cardiovascular and especially locomotor activity were affected. The reduction of locomotor activity was manifested in the dark phase in the evenings following the stress. ACTH pretreatment did not alter the cardiovascular or locomotor responses during the stress; however, it delayed the recovery of these functions subsequent to cessation of the stress.

Acknowledgments

This work was supported in part by a Senior Development Award 0130102N (L.I.S.) from the American Heart Association and NIH grant NS 44218 (E.L.S.).

References

- 1 Selye H: The Stress of Life. New York, McGraw Hill, 1975.
- 2 Chrousos GP, Gold PW: The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 1992;267:1244–1252.
- 3 McEwen BS: Protective and damaging effects of stress mediators. *N Engl J Med* 1998; 338:171–179.
- 4 Goldstein DS: Stress, Catecholamines and Cardiovascular Disease. Oxford, Oxford University Press, 1995.
- 5 Axelrod J, Reisine TD: Stress hormones: their interaction and regulation. *Science* 1984;224:452–459.
- 6 Kvetnansky R, Mikulaj L: Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. *Endocrinology* 1970;87:738–743.
- 7 Kvetnansky R, Pacak K, Fukuhara K, et al: Sympathoadrenal system in stress. Interaction with the hypothalamic-pituitary-adrenocortical system. *Ann NY Acad Sci* 1995; 771:131–158.
- 8 Goldstein DS: Catecholamines and stress. *Endocr Regul* 2003;37:69–80.
- 9 Aguilera G: Regulation of pituitary ACTH secretion during chronic stress. *Front Neuroendocrinol* 1994;15:321–350.
- 10 Nankova B, Kvetnansky R, Hiremagalur B, Sabban B, Rusnak M, Sabban EL: Immobilization stress elevates gene expression for catecholamine biosynthetic enzymes and some neuropeptides in rat sympathetic ganglia: effects of adrenocorticotropin and glucocorticoids. *Endocrinology* 1996;137:5597–5604.
- 11 Nankova BB, Kvetnansky R, Sabban EL: Adrenocorticotrophic hormone (MC-2) receptor mRNA is expressed in rat sympathetic ganglia and up-regulated by stress. *Neurosci Lett* 2003;344:149–152.
- 12 Versteeg DH, Van Bergen P, Adan RA, De Wildt DJ: Melanocortins and cardiovascular regulation. *Eur J Pharmacol* 1998;360:1–14.
- 13 Whitworth JA, Hewitson TD, Ming L, et al: Adrenocorticotrophin-induced hypertension in the rat: haemodynamic, metabolic and morphological characteristics. *J Hypertens* 1990;8:27–36.
- 14 Kawabe T, Chitravanshi VC, Kawabe K, Sapru HN: Cardiovascular effects of adrenocorticotropin microinjections into the rostral ventrolateral medullary pressor area of the rat. *Brain Res* 2006;1102:117–126.
- 15 Brown S, Chitravanshi VC, Sapru HN: Cardiovascular actions of adrenocorticotropin microinjections into the nucleus tractus solitarius of the rat. *Neuroscience* 2006;143: 863–874.
- 16 Torpy DJ, Mullen N, Ilias I, Nieman LK: Association of hypertension and hypokalemia with Cushing's syndrome caused by ectopic ACTH secretion: a series of 58 cases. *Ann NY Acad Sci* 2002;970:134–144.

- 17 Kvetnansky R, Fukuhara K, Pacak K, Cizza G, Goldstein DS, Kopin IJ: Endogenous glucocorticoids restrain catecholamine synthesis and release at rest and during immobilization stress in rats. *Endocrinology* 1993; 133:1411–1419.
- 18 McDougall SJ, Lawrence AJ, Widdop RE: Differential cardiovascular responses to stressors in hypertensive and normotensive rats. *Exp Physiol* 2005;90:141–150.
- 19 Chen X, Herbert J: Regional changes in c-fos expression in the basal forebrain and brainstem during adaptation to repeated stress: correlations with cardiovascular, hypothalamic and endocrine responses. *Neuroscience* 1995;64:675–685.
- 20 Ely DL: Organization of cardiovascular and neurohumoral responses to stress. Implications for health and disease. *Ann NY Acad Sci* 1995;771:594–608.
- 21 Grippio AJ, Beltz TG, Johnson AK: Behavioral and cardiovascular changes in the chronic mild stress model of depression. *Physiol Behav* 2003;78:703–710.
- 22 Valles A, Marti O, Armario A: Long-term effects of a single exposure to immobilization: A C-fos mRNA study of the response to the homotypic stressor in the rat brain. *J Neurobiol* 2006;66:591–602.
- 23 Farah VM, Joaquim LF, Morris M: Stress cardiovascular/autonomic interactions in mice. *Physiol Behav* 2006;89:569–575.
- 24 Lamprecht F, Williams RB, Kopin IJ: Serum dopamine-beta-hydroxylase during development of immobilization-induced hypertension. *Endocrinology* 1973;92:953–956.
- 25 Holson RR, Scallet AC, Ali SF, Turner BB: 'Isolation stress' revisited: isolation-rearing effects depend on animal care methods. *Physiol Behav* 1991;49:1107–1118.
- 26 Brockway BP, Mills PA, Azar SH: A new method for continuous chronic measurement and recording of blood pressure, heart rate and activity in the rat via radio-telemetry. *Clin Exp Hypertens A* 1991;13:885–895.
- 27 Masineni SN, Chander PN, Singh GD, Powers CA, Stier CT Jr: Male gender and not the severity of hypertension is associated with end-organ damage in aged stroke-prone spontaneously hypertensive rats. *Am J Hypertens* 2005;18:878–884.
- 28 Sepehrdad R, Chander PN, Oruene A, Rosenfeld L, Levine S, Stier CT Jr: Amiloride reduces stroke and renal injury in stroke-prone hypertensive rats. *Am J Hypertens* 2003;16: 312–318.
- 29 Sabban EL, Nankova BB, Serova LI, Kvetnansky R, Liu X: Molecular regulation of gene expression of catecholamine biosynthetic enzymes by stress: sympathetic ganglia versus adrenal medulla. *Ann NY Acad Sci* 2004;1018:370–377.
- 30 Serova LI, Nankova BB, Feng Z, Hong JS, Hutt M, Sabban EL: Heightened transcription for enzymes involved in norepinephrine biosynthesis in the rat locus coeruleus by immobilization stress. *Biol Psychiatry* 1999;45: 853–862.
- 31 Stier CT Jr, Serova LI, Singh G, Sabban EL: Stress triggered rise in plasma aldosterone is lessened by chronic nicotine infusion. *Eur J Pharmacol* 2004;495:167–170.
- 32 Sabban EL, Liu X, Serova L, Gueorguiev V, Kvetnansky R: Stress triggered changes in gene expression in adrenal medulla: transcriptional responses to acute and chronic stress. *Cell Mol Neurobiol* 2006;26:845–854.
- 33 McMahon A, Kvetnansky R, Fukuhara K, Weise VK, Kopin IJ, Sabban EL: Regulation of tyrosine hydroxylase and dopamine beta-hydroxylase mRNA levels in rat adrenals by a single and repeated immobilization stress. *J Neurochem* 1992;58:2124–2130.
- 34 Vahl TP, Ulrich-Lai YM, Ostrander MM, et al: Comparative analysis of ACTH and corticosterone sampling methods in rats. *Am J Physiol Endocrinol Metab* 2005;289:E823–E828.
- 35 Hauger RL, Lorang M, Irwin M, Aguilera G: CRF receptor regulation and sensitization of ACTH responses to acute ether stress during chronic intermittent immobilization stress. *Brain Res* 1990;532:34–40.
- 36 Bartolomucci A, Palanza P, Costoli T, et al: Chronic psychosocial stress persistently alters autonomic function and physical activity in mice. *Physiol Behav* 2003;80:57–67.
- 37 Pardon MC, Gould GG, Garcia A, et al: Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: implications for susceptibility to stress-related neuropsychiatric disorders. *Neuroscience* 2002;115:229–242.
- 38 Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S: Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proc Natl Acad Sci USA* 2005;102:9371–9376.
- 39 Kim KS, Han PL: Optimization of chronic stress paradigms using anxiety- and depression-like behavioral parameters. *J Neurosci Res* 2006;83:497–507.
- 40 Van Bergen P, Kleijne JA, De Wildt DJ, Versteeg DH: Different cardiovascular profiles of three melanocortins in conscious rats; evidence for antagonism between gamma 2-MSH and ACTH-(1–24). *Br J Pharmacol* 1997;120:1561–1567.
- 41 Fraser TB, Turner SW, Mangos GJ, Ludbrook J, Whitworth JA: Comparison of telemetric and tail-cuff blood pressure monitoring in adrenocorticotrophic hormone-treated rats. *Clin Exp Pharmacol Physiol* 2001;28:831–835.
- 42 Cole CR, Foody JM, Blackstone EH, Lauer MS: Heart rate recovery after submaximal exercise testing as a predictor of mortality in a cardiovascularly healthy cohort. *Ann Intern Med* 2000;132:552–555.
- 43 Morshedi-Mebodi A, Larson MG, Levy D, O'Donnell CJ, Vasan RS: Heart rate recovery after treadmill exercise testing and risk of cardiovascular disease events (The Framingham Heart Study). *Am J Cardiol* 2002;90: 848–852.
- 44 Schneider GM, Jacobs DW, Gevirtz RN, O'Connor DT: Cardiovascular haemodynamic response to repeated mental stress in normotensive subjects at genetic risk of hypertension: evidence of enhanced reactivity, blunted adaptation, and delayed recovery. *J Hum Hypertens* 2003;17:829–840.
- 45 Dallman MF, Strack AM, Akana SF, et al: Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front Neuroendocrinol* 1993;14:303–347.
- 46 Grünfeld J: Glucocorticoids in blood pressure regulation. *Horm Res* 1990;34:111–113.
- 47 Whitworth JA, Brown MA, Kelly JJ, Williamson PM: Mechanisms of cortisol-induced hypertension in humans. *Steroids* 1995;60:76–80.
- 48 Darlington DN, Kaship K, Keil LC, Dallman MF: Vascular responsiveness in adrenalectomized rats with corticosterone replacement. *Am J Physiol* 1989;256:H1274–H1281.
- 49 van den Buuse M, van Acker SA, Flutterm MF, de Kloet ER: Involvement of corticosterone in cardiovascular responses to an open-field novelty stressor in freely moving rats. *Physiol Behav* 2002;75:207–215.
- 50 Serova LI, Gueorguiev V, Cheng SY, Sabban EL: Adrenocorticotrophic hormone elevates gene expression for catecholamine biosynthesis in rat superior cervical ganglia and locus coeruleus by an adrenal independent mechanism. *Neuroscience* 2008;153:1380–1389.