Prolonged exercise increases peripheral plasma ACTH, CRH, and AVP in male athletes

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Inder, W. J., J. Hellemans, M. P. Swanney, T. C. R. Pickett, and R. A. Donald. Prolonged exercise increases peripheral plasma ACTH, CRH, and AVP in male athletes. J. Appl. Physiol. 85(3): 835–841, 1998.—We wished to determine whether the increased ACTH during prolonged exercise was associated with changes in peripheral corticotropin-releasing hormone (CRH) and/or arginine vasopressin (AVP). Six male triathletes were studied during exercise: 1 h at 70% maximal oxygen consumption, followed by progressively increasing work rates until exhaustion. Data obtained during the exercise session were compared with a nonexercise control session. Venous blood was sampled over a 2-h period for cortisol, ACTH, CRH, AVP, renin, glucose, and plasma osmolality. There were significant increases by ANOVA on log-transformed data in plasma cortisol (P < 0.001), CRH (P < 0.001), and AVP (P < 0.03) during exercise compared with the control day. A variable increase in AVP was observed after the period of high-intensity exercise. Plasma osmolality rose with exercise (P < 0.001) and was related to plasma AVP during submaximal exercise (P < 0.03) but not with the inclusion of data that followed the high-intensity exercise. This indicated an additional stimulus to the secretion of AVP. The mechanism by which ACTH secretion occurs during exercise involves both CRH and AVP. We hypothesize that high-intensity exercise favors AVP release and that prolonged duration favors CRH release.

Cortisol; osmolality; glucose; renin; adrenocorticotropic hormone; corticotropin-releasing hormone; arginine vasopressin

The response of the hypothalamic-pituitary-adrenal (HPA) axis to acute exercise has been extensively studied. Exercise to >60% of maximal O2 consumption (V\textsuperscript{O2max}) results in ACTH and cortisol release in proportion to the exercise intensity (16). Highly trained athletes show a similar degree of stimulation of the HPA axis at the same relative exercise intensity (%V\textsuperscript{O2max}), although in absolute terms they require a greater workload compared with untrained individuals (22).

In attempting to examine the mechanism of the ACTH stimulation during acute exercise in humans, some investigators have measured peripheral plasma levels of the major ACTH secretagogues corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) (22, 37). A previous study from this department showed no increase in peripheral CRH levels in response to 15 min of high-intensity exercise (90% V\textsuperscript{O2max}), whereas ACTH and AVP rose synchronously (37). This confirmed the results of Luger et al. (22), who also failed to detect a rise in peripheral CRH, although the assay they employed lacked sensitivity. In the exercising horse, synchronous pulses of AVP and ACTH are noted, without change in CRH levels, as measured in pituitary venous effluent (3). There is considerable variability in the observed increase in cortisol, ACTH, and AVP induced by acute exercise; this variability relates to the suppressibility of this response by dexamethasone (28, 29). Subjects with a greater AVP response to intense exercise do not have complete ACTH suppressibility when exercise is preceded by a 4-mg dose of dexamethasone (28, 29). These observations support the concept that, during short-duration, high-intensity exercise, AVP may become the more important ACTH secretagogue, potentiating CRH-induced ACTH secretion. The data in studies of horses add weight to the studies of humans; the latter have the limitation of peripheral plasma measurements.

During prolonged exercise of lesser intensity, changes in the HPA axis also occur (16). To our knowledge, only two previous human studies have measured plasma CRH in protocols in which exercise was maintained for 1 h or more (14, 34). After exercise at 50% V\textsuperscript{O2max} until exhaustion, an increase in CRH, ACTH, and cortisol was seen that was associated with a fall in plasma glucose to <3.3 mmol/l (34). No activation of the HPA axis was seen when glucose was kept constant by using an intravenous glucose infusion during the exercise (34). In a further study, in which plasma CRH, β-endorphin, and cortisol were measured after a 1-h run, CRH was clearly elevated compared with a control day (14).

By using an original protocol that combined prolonged submaximal exercise followed by high-intensity exercise, the present study was designed to determine whether the expected increases in ACTH and cortisol during exercise were associated with changes in peripheral CRH and/or AVP and to examine glucose levels, plasma osmolality, and volume status as possible contributing factors in mediating the response. Endurance athletes, such as triathletes, duathletes, and marathon runners, repeatedly exercise for prolonged periods of time to train for their events. Athletes were selected as subjects because of the physically demanding nature of the exercise protocol and because of the relevance of the basal ACTH hypersecretion previously documented (17, 22).

Materials and Methods

Subjects

Six highly trained male triathletes, ages 19–43 yr, took part in the study. The mean body mass index was 23.0 ± 0.5 kg/m\textsuperscript{2}. The average weekly training undertaken by the athletes was swimming 11 ± 4 km, cycling 230 ± 21 km, and running 63 ± 5 km. No subject was taking any medications. Written informed consent was obtained from each subject, and the study was approved by the Ethics Committee of the Southern Regional Health Authority.
and after exercise, and sweat volume was calculated as
the subject wearing only cycle shorts) was measured before
V˙O2max, as calculated from his V˙O2max test. V˙O2 was measured
each athlete was required to exercise for 60 min at 70%
warm-up at 150 W commenced at 1050. Beginning at 1100,
5, 10, 15, 30, and 60 min, and the power output
was titrated to maintain the V˙O2max at 70 ± 3% V˙O2max. At 60
min, the power output was increased by 25 W every 2 min
until the athlete was exhausted. The athletes were instructed
to maintain a constant speed of ~80 rpm throughout
the exercise period. Recovery was at 150 W for as long as the
athlete required. Additional venous blood samples were col-
clected at t = 0, 30, 60, 75, and 90 min. Sampling was carried
out while athletes were in a seated posture, either at rest or
during the exercise on the cycle ergometer. Over the 90-min
exercise and/or sampling period, each athlete ingested 200 ml
water every 20 min (total 900 ml over the 90 min). A final
urine sample was collected at t = 90 min. Dry body mass (with
the subject wearing only cycle shorts) was measured before
and after exercise, and sweat volume was calculated as
follows: (loss in body mass + mass of fluid ingested) –
(respiratory water loss + urine volume).
On the nonexercise control day, a 21-gauge butterfly can-
nula was used to sample the venous blood. The athletes
rested in a seated position, and, as on the exercise day, the
total fluid intake over the 90-min period was 900 ml water.
The timing of blood and urine collection was identical to
timing on the exercise day.

Equipment

Breath-by-breath data for expiratory flow, O2, and CO2
were monitored by using a SensorMedics 2900 metabolic cart.
Heart rate was monitored by using a Marquette Max-1
electrocardiogram stress system, with the heart rate signal
interfaced into the metabolic cart. All exercise tests were
performed on a SensorMedics 800 Ergometer that had been
modified to fit a competitive cyclist's seat, and each athlete
used his own pedals. Expiratory volume was obtained by
integration of the flow signal.

All parameters were calibrated before each testing session.
The flow calibration was verified by using a SensorMedics 3-liter calibration syringe. A two-point calibration of O2 and
CO2 was achieved by using two Beta standard gas mixtures:
26 ± 0.02% O2-balance N2, and 16 ± 0.02% O2-4.1% CO2-
balance N2. Ambient conditions for room temperature, baro-
metric pressure, and relative humidity were entered into the
metabolic cart before each calibration and exercise test, and
data were recorded after each exercise test. Results were
calculated after averaging data over a 30-s interval.

Assays

Cortisol was measured by ELISA (20). ACTH was mea-
sured by immunoradiometric assay (Nicols Institute). The
assay coefficients of variation are 3.0% (intra-assay) and 7.8%
(interassay), and the sensitivity is 0.7 pmol/l. CRH was
measured by radiodimmunoassay in methanol extracts of
plasma samples (1 ml), as previously described (9). The
antiserum (Y2B0) was a gift from Professor P. J. Lowry,
Department of Physiology and Biochemistry, University of
Reading, UK. Assay coefficients of variation are 10% (intra-
assay) and 11% (interassay) at 3.5 pmol CRH/l, and the
detection limit (at 95% confidence level) is 0.2 pmol/l. AVP was
measured by radiodimmunoassay with the use of acetonitrile
extracts of plasma (2:1, vol/vol),125I-labeled AVP purified by
using HPLC in a NH2-HCO3 buffer eluted with a gradient of
12–48% 2-propanol at 1%/min, and a previously validated
antiserum (17). The coefficients of variation are 9.7% (intra-
assay) and 10.8% (interassay) at 4 pmol AVP/l, and the
detection limit (at 95% confidence level) is 0.3 pmol/l. Plasma
renin activity was measured by a trapping assay (26). Other
biochemical parameters measured were plasma and urine
osmolality and plasma glucose.

Statistical Analysis

Hormone and metabolic data were log transformed to
stabilize the variance before analysis by using two-way
repeated-measures ANOVA, with study condition (exercise
vs. control) being the between factor and with time being the
within factor. Log-transformed hormonal and biochemical
measures were examined for overall changes between
30 and 90 min. This range included baseline, exercise at submaxi-
mal and maximal intensities, and recovery. Where significant
changes were observed with ANOVA, Bonferroni post hoc
analysis was used to detect differences from baseline values
(t = −30 min) and control time-matched data as appropriate.
General linear models were used to test the associations

Exercise Protocol

V˙O2max test. V˙O2max was assessed for each athlete during his
initial visit to the laboratory. A standard testing protocol,
using a cycle ergometer (see Equipment), was employed for
all athletes. After a 4-min warm-up period was performed at
150 W, the workload was increased to 250 W for 4 min and
then by 50-W increments at 4-min intervals until exhaustion.
The workload changes were achieved automatically under
the software control of the Sensormedics 2900 metabolic cart.

Study day exercise test. After the V˙O2max test, each athlete
attended the laboratory on two separate occasions for an
exercise day and a nonexercise control day. Training was
forbidden on the mornings before testing took place. The
athletes were required to eat a standard diet, with breakfast
at 0700. Fluid intake was controlled (500 ml water with
breakfast along with 250 ml orange juice, and a further 200
ml of water at 0930). The athlete reported to the laboratory at
1000. To facilitate venous blood sampling during exercise, a
13-gauge antecubital fossa long line was inserted with the use
of local anesthesia; after the insertion, the athlete rested
quietly.

A timeline for the exercise and/or sampling protocol is
outlined in Fig. 1. Initial baseline venous blood and urine
samples were collected at 1030 (t = −30 min). A 10-min
warm-up at 150 W commenced at 1050. Beginning at 1100,
each athlete was required to exercise for 60 min at 70%
V˙O2max, as calculated from his V˙O2max test. V˙O2 was measured
at time (t) = 5, 10, 15, 30, and 60 min, and the power output
was titrated to maintain the V˙O2max at 70 ± 3% V˙O2max. At 60
min, the power output was increased by 25 W every 2 min
until the athlete was exhausted. The athletes were instructed
to maintain a constant speed of ~80 rpm throughout
the exercise period. Recovery was at 150 W for as long as the
athlete required. Additional venous blood samples were col-
clected at t = 0, 30, 60, 75, and 90 min. Sampling was carried
out while athletes were in a seated posture, either at rest or
during the exercise on the cycle ergometer. Over the 90-min
exercise and/or sampling period, each athlete ingested 200 ml
water every 20 min (total 900 ml over the 90 min). A final
urine sample was collected at t = 90 min. Dry body mass (with
the subject wearing only cycle shorts) was measured before
and after exercise, and sweat volume was calculated as
RESULTS

These athletes were extremely fit, as demonstrated by mean VO$_{2\text{max}}$ of 69.0 ± 0.7 ml·kg$^{-1}$·min$^{-1}$. Between 0 and 60 min, the mean intensity of exercise was maintained between 69 and 72% VO$_{2\text{max}},$ and it rose to 96.1 ± 2.2% VO$_{2\text{max}}$ during the final period of increasing exercise intensity. During the exercise session, the mean sweat volume was 1,670 ± 97 ml while the body mass of the athletes fell from 74.3 ± 1.2 to 73.2 ± 1.1 kg (P < 0.001).

Figure 2 outlines the levels of cortisol, ACTH, CRH, and AVP during the 90-min study period on the exercise and control days. Log-transformed levels of each of these hormones were not significantly different at baseline on the two occasions. Between the two study conditions, exercise vs. control, there was a significantly different time course of hormone response (time × study condition interaction) for log cortisol (F = 5.34, P = 0.002), log ACTH (F = 19.59, P < 0.001), log CRH (F = 20.91, P < 0.001), and log AVP (F = 3.09, P < 0.03) over the 2-h period between −30 and 90 min. These data indicate a significant rise in each of these hormones during the exercise protocol. Further post hoc analysis was undertaken by using the Bonferroni t-test as described in the MATERIALS AND METHODS. Plasma ACTH was significantly different from baseline (P < 0.01) and time-matched control data (P < 0.001) by t = 30 min and showed a large peak at t = 75 min. Cortisol, which had also begun to rise by t = 30 min (P < 0.01 compared with time-matched control), became significantly different from baseline by t = 60 min (P < 0.01) and continued to increase up to t = 90 min. Plasma CRH was significantly elevated at the 60-min time point and beyond, both compared with baseline and with control data (P < 0.001 for each). Plasma AVP was significantly different from baseline (P < 0.05) and time-matched control data (P < 0.01) only at t = 75 min, after the period of high-intensity exercise.

Plasma glucose (Fig. 3A) was similar at baseline under the two study conditions. During exercise, glucose levels fell between 0 and 60 min and subsequently rose between 60 and 75 min, whereas no significant changes occurred over the equivalent times on the control day. Across all time points, ANOVA on the log-transformed data revealed a significant time × study condition interaction (F = 5.23, P = 0.002). The Bonferroni t-test revealed a significantly lower glucose at t = 60 min (4.3 ± 0.35 vs. 4.9 ± 0.1 mmol/l; P < 0.01). Although no subject became frankly hypoglycemic, one developed a level of 3.3 mmol/l at 60 min, whereas the other athletes maintained levels greater than or equal to 3.5 mmol/l throughout. Plasma renin activity (Fig. 3B) was not significantly different at baseline. There was a significant effect of exercise on log plasma renin activity (F = 17.38, P < 0.001), with the Bonferroni t-test confirming significantly elevated levels at t = 60 and 90 min compared with baseline and time-matched control data (P < 0.001). Plasma osmolality (Fig. 3C) was similar at baseline. Changes in log plasma osmolality during exercise were highly significant overall (F = 20.56, P < 0.001). The Bonferroni t-test indicated that there was a significant increase from baseline during exercise between t = 30 and 75 min (P < 0.001), a significant decrease from baseline during the control
but, during high-intensity exercise, AVP becomes more dependent on an additional stimulus.

Urine osmolality did not show a significant exercise-induced change (exercise day: t = −30 min: 553 ± 137 mosmol/kgH₂O; t = 90 min: 499 ± 120 mosmol/kgH₂O; control day: t = −30 min: 727 ± 130 mosmol/kgH₂O, t = 90 min: 402 ± 108 mosmol/kgH₂O; ANOVA on log-transformed data, change over time, F = 3.74, P > 0.1; time × study condition interaction F = 1.83, P > 0.2).

**DISCUSSION**

The results of this study confirm that prolonged submaximal exercise is stimulatory to ACTH and cortisol secretion. This is associated with significant increases in peripheral CRH, compared with nonexercise conditions with similar fluid intake. Although glucose levels fell slightly during the period of prolonged exercise, the elevation in CRH in this study was not associated with frank hypoglycemia. The observed rise in peripheral CRH is in contrast to the situation in acute, nearly maximal exercise, in which, most studies, show CRH fails to change from baseline (22, 37). There is, however, one study that reported an increase in CRH immediately after a short period of intense exercise, as per the Bruce protocol, but there were no control data (8). Our results are consistent with those of Harte et al. (14), who observed an increase in peripheral CRH after a 1-h run. Although there is considerable debate concerning the relevance of peripheral measurement of CRH, a central source of the rise may be suggested by a positive correlation between CRH and mood enhancement during the exercise (14). Whereas basal CRH, as measured in peripheral blood, is derived largely from extrahypothalamic sources (31), the incremental rise seen during the stress of hypoglycemia is thought to represent hypothalamic secretion (9, 31).

Prolonged exercise being stimulatory to CRH secretion would be consistent with the hypothesis that highly trained athletes have increased levels of hypothalamic CRH (22). Repeated bouts of prolonged exercise, such as occur during their normal training routines, may result in chronic elevation in central CRH levels, leading to the observed increase in basal ACTH (17, 22).

Previous studies from this department have documented increases in peripheral CRH during the stress of abdominal surgery (7) and myocardial infarction (6), but not during ethanol-induced nausea (18) or cardioversion and electroconvulsive therapy (12), where AVP appears to be the predominant mediator of the acute rise in ACTH. It is acknowledged that small increases in CRH at the level of the hypothalamic-hypophysial portal (HHP) system will not give rise to changes in peripheral levels caused by the dilution effect. Nevertheless, studies examining HHP blood or pituitary venous blood in higher mammals, such as sheep (10) and horses (2), have suggested that several forms of acute stress activate ACTH secretion, predominantly via AVP, with CRH having a more permissive role.

Under normal conditions, plasma AVP is closely correlated with plasma osmolality in a linear fashion, although, when pronounced changes in osmolality oc-
cur, a parabolic model produces a better fit (13). In our study, during prolonged submaximal exercise, AVP showed a small increase that just failed to reach statistical significance compared with baseline at t = −30 min. The extent of this rise was in keeping with that expected from the changes that occurred in plasma osmolality. However, where a short period of maximal exercise was superimposed on the prolonged exercise, a further rise in AVP was noted; in some subjects, this increase appeared to be greater than expected from plasma osmolality changes alone. This rise in AVP was associated with an additional increase in ACTH and, later, in cortisol, and the increase varied considerably between subjects.

Recent evidence suggests that subjects may be divided into two groups based on whether their HPA-axis response to high-intensity exercise is completely or incompletely suppressed by 4 mg of dexamethasone (28, 29). The nonsuppressors also have greater exercise-induced secretion of AVP, ACTH, and cortisol after placebo (28, 29). Thus any population will have in it both suppressors and nonsuppressors, which may explain the variability in the hormonal response to exercise, particularly in regard to AVP. With only six subjects, our study lacks the statistical power to differentiate between these groups, and we did not set out to do this. However, it is interesting to note that the variability in AVP response was predominantly seen at the 75-min time point after the period of intense exercise and that, during the submaximal exercise, the increase in AVP was quite consistent between subjects in proportion to the changing plasma osmolality. This observation may imply that the variability in HPA-axis response to intense exercise is largely related to the magnitude of the AVP increment. One might hypothesize, therefore, that the observed difference in HPA-axis response between suppressors and nonsuppressors after intense exercise would not be seen with prolonged submaximal exercise. This deserves future study with a larger group of subjects.

The results during the maximal exercise, with AVP and ACTH increasing synchronously, are similar to those previously reported for humans (37) and horses (3). The mechanism of this rise is unclear. Convertino et al. (4) attributed the exercise-induced rise in AVP to plasma osmolality changes, and, using second-order correlation, AVP failed to show a significant relationship between plasma volume or plasma renin activity. Their study demonstrated a curvilinear response of both osmolality and AVP to a protocol of graded exercise in which the subjects developed mean plasma osmolality levels of >300 mosmol/kg H2O. In a further study of plasma AVP during exercise (35), changes in AVP were not correlated with changes in plasma osmolality or plasma volume over all observations, but changes in AVP did show a positive correlation with work intensity. Although increases in plasma catecholamines are known to occur during exercise (5), human evidence does not support a role for peripheral catecholamines in promoting ACTH secretion (24). However, it is possible that activation of central α1-noradrenergic mechanisms, which are known to stimulate ACTH predominantly via AVP secretion (1, 21), may be involved.

It is not possible in our study to distinguish AVP arising from parvocellular vs. magnocellular sources. However, it is clear from several previous studies, both in the horse (19) and in humans (23, 30), that AVP from a magnocellular source may still stimulate ACTH secretion. Data from rats indicate that neurons derived from the magnocellular region of the paraventricular nucleus pass through the median eminence and release vasopressin, which may act directly on the anterior pituitary (15). In addition, variations in blood flow in the HHP system may also result in the delivery of magnocellular-derived AVP to the anterior pituitary (27).

Exercise may be a form of stress where the intensity and/or duration has a differential effect on factors that mediate ACTH secretion, either with CRH predominating or with CRH and AVP assuming equal importance during prolonged, submaximal exercise and with AVP becoming more important during short-term, nearly maximal exercise. A previous study (32), in which 20 min of exercise were performed, demonstrated that exercise after 4 h of a 1 μg/kg·h1 infusion of ovine CRH induced a similar incremental rise in ACTH in response to exercise during a saline infusion, despite elevated baseline cortisol and ACTH levels and saturated corticotropin CRH receptors. The authors concluded that other factors, mostly likely AVP, act in concert with CRH to mediate ACTH release during acute exercise (32).

The present study utilized a unique protocol in which highly trained athletes sequentially performed prolonged submaximal exercise and high-intensity exercise. Is it possible that the HPA-axis response to the high-intensity exercise was altered by the preceding period of exercise? Theoretically, one could postulate either an enhancement or an attenuation of the ACTH and cortisol response, depending on whether the dominant factor at the commencement of the period of high-intensity exercise is increased baseline CRH or cortisol. In fact, maximal ACTH and cortisol levels in response to the intense exercise in the present study were similar to those of other studies of acute exercise alone, including a previous study from this department (22, 28, 29, 37).

It is also possible that differences between subjects in the lactate threshold could influence the extent of the HPA-axis response to submaximal exercise, because plasma lactate correlates with ACTH and cortisol secretion at 70% VO2max and above (22). Endurance training increases the relative exercise intensity that results in the accumulation of blood lactate (25, 33, 36), whereas the optimal training intensity is at or just above the lactate threshold (11, 33, 36). Although we did not formally measure lactate as part of the present exercise protocol, preliminary testing on athletes indicated that our chosen exercise intensity of 70% VO2max was likely to be at or just below the lactate threshold for this group (J. Hellemans, unpublished observations). This is in agreement with an earlier study that demonstrated that the onset of plasma lactate accumulation (defined in this study as a change in blood lactate of >1 mmol/l
above baseline) in a group of trained athletes was up to 78.9% \( \overline{V_{O_2}} \text{max} \) (11). Our data for AVP, ACTH, and cortisol at \( t = 60 \) min, the end of the submaximal exercise, show considerably less variability than at the \( t = 75 \) min time point on completion of the high-intensity exercise. This indicates that any difference in lactate threshold between the athletes had relatively little impact on the HPA-axis response variability, compared with the stimulus brought about by the high-intensity exercise.

In conclusion, prolonged, submaximal exercise is associated with significant rises in peripheral CRH that are not associated with hypoglycemia. The rise in AVP is consistent with the plasma osmolality changes that occur. High-intensity exercise is associated with a further elevation in ACTH, combined with an increase in AVP, which in some subjects is out of proportion to osmolality changes. It is proposed that the mechanism by which activation of the HPA axis occurs during exercise may involve the hypothalamic secretion of both CRH and AVP, with high-intensity exercise favoring AVP release and prolonged-duration exercise favoring CRH release.

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