Chromogranin A Concentrations in Plasma of Physically Active Men after Acute Exercise

To the Editor:

Chromogranin A is an acidic monomeric protein that is present along with catecholamines in storage vesicles in sympatb-chromaffin cells in the adrenal medulla and elsewhere (1–3). Chromogranin A is coreleased from these vesicles along with catecholamines (1, 4). Recently, measurement of chromogranin A has been used to investigate patients with pheochromocytoma and other forms of hypertension (5). Before the commercial availability of chromogranin A assays, screening of hypertensive patients thought to have pheochromocytoma was based on measurement of urinary concentrations of catecholamines and their metabolites (6) or measurement of serum catecholamines (7, 8). Because catecholamine concentrations in plasma are greatly influenced by posture, physical activity, stress, and numerous medications (9), screening tests for pheochromocytomas that rely on measurement of urine or plasma catecholamine and their metabolites must take into account and exclude these interfering factors.

Plasma chromogranin A concentrations are not significantly influenced by posture, venipuncture, or many medications that interfere with measurement of catecholamines or their metabolites (10). The effect of physical activity on plasma chromogranin A concentrations has not been fully investigated, but physical activity is known to increase concentrations of plasma catecholamines. We studied the effect of strenuous physical activity on plasma chromogranin A concentrations in physically active male volunteers, specifically evaluating the rise in chromogranin A after such exercise for comparison with previously reported values for patients with essential hypertension and pheochromocytomas.

Eight men, ages 20–42 years (mean ± SD, 26.6 ± 7.1 years), participated in the study. They weighed 58–94.5 kg (mean ± SD, 70.6 ± 13.0 kg) and were 154–185.4 cm tall (mean ± SD, 192.9 ± 10.4 cm). Although serum creatinine concentrations were not measured, no volunteers had a history of renal impairment. All studies were performed between 0830 and 1000 after an overnight fast.

All exercises were performed on a motorized treadmill (Marathon 7000; Cal-Med, Brea, CA). Ventilatory measurements during exercise were made on a breath-by-breathe basis, and values were averaged and reported for each 15-s interval. Subjects breathed through a Model 2700B valve (Hans-Rudolph, Kansas City, MO; dead space 90 mL). Expired air was sampled just distal to the valve and was routed via capillary tubing into a mass spectrometer (Model 1100; Perkin-Elmer, Norwalk, CA) at the rate of 45–60 mL/min.

The Hans-Rudolph valve was connected to a pneumotachograph (Model 50MCC2-2; Meriam, Cleveland, OH) that was connected to a differential-pressure transducer and a carrier de-modulator (Model CD-15; Validyne, Northridge, CA). Analog signals, based on sampling every 15 ms, were digitized (Keithley System Model 570; Data Acquisition and Control, Cleveland, OH). The digitized signals were sent to a computer (IBM-AT clone; Micro Express 286, Santa Ana, CA), where metabolic measurements were calculated in real time and analysed.

The flow signal from the pneumotachograph was integrated to determine expired minute ventilation (Ve), oxygen uptake (Vo2), and carbon dioxide production (VCO2). Respiratory exchange ratios were calculated from Ve and exhaled gas measurements. Cardiac rhythm was continuously monitored with a simultaneous three-lead electrocardiogram (Model ECG 3A; Brentwood Instruments, Torrance, CA).

Exercise testing was performed by using the Bruce multistage protocol with a 3-min preexercise baseline (standing) stage followed by a 3-min warm-up at 0.765 m/s 5% grade (11). An electrocardiogram strip was taken 20 s before the end of each minute of the test. The Borg scale of rating of perceived exertion (RPE) (12), indicated with finger signals from 6 to 20, was obtained near the beginning and end of each stage. The mean total exercise time, excluding warm-up, was 12.5–17 min (mean ± SD, 14.4 ± 1.4 min). All eight volunteers completed at least four stages of the Bruce protocol. Perceived exertion at the conclusion of exercise was 19–20.

Blood was removed for hormone measurements within 1 min after exercise. Peak VO2 (VO2max) was obtained from measurements during the maximum power level achieved. After 30 min of recumbency, blood was removed from the antecubital vein via a 16-gauge indwelling catheter for measurement of baseline plasma concentrations of chromogranin A. Blood was then removed immediately after exercise and at 15, 30, 60, 90, and 180 min after exercise. The plasma was separated and stored at −70°C until assayed. Plasma chromogranin A was measured by radioimmunoassay by using 125I-labeled chromogranin A and rabbit antisera to purified chromogranin A (13). Separation of bound from free chromogranin A was accomplished by using a second antibody (goat anti-rabbit γ-globulin). The lower limit of sensitivity of the assay is 1.5 μg/L at 90% B/B0. Intra-assay variation was <8% and interassay variation was <12%.

Serum lactic acid concentration was measured by standard laboratory techniques (14) in all subjects within 30 s after the completion of exercise.

Statistical analysis was performed...
by analysis of variance (repeated-measures design) and Student’s t-test was used for paired and grouped observations. The level of significance was ≤0.05.

The mean maximum heart rate (± SD) was 190.9 ± 7.1 beats/min, which was 95–102% of the maximum rate predicted for the volunteers. Baseline and peak systolic blood pressure were 121.0 ± 13.4 and 176.0 ± 13.8 mmHg, respectively. Corresponding baseline and diastolic blood pressure were 80.3 ± 6.0 and 73.3 ± 8.8 mmHg, respectively. VO_{max} was 53.5 ± 7.7 mL·kg^{-1}·min^{-1}. Peak V_{O_2} was 145.5 ± 29.2 L/min. The respiratory exchange ratio at the time of maximum exertion was 1.24 ± 0.07. Serum lactic acid concentrations immediately after exercise ranged from 15.8 to 18.6 mmol/L (17.0 ± 0.85 mmol/L).

Plasma chromogranin A concentration rose from a baseline value of 40.94 ± 10.23 μg/L to a peak of 55.98 ± 3.54 μg/L (P <0.005) at 15 min postexercise (Figure 1). Baseline plasma chromogranin A concentration was similar to that reported in normal healthy subjects (45.0 ± 3.0 μg/L) (5). A peak chromogranin A concentration at 2 min after high-intensity exercise was reported by Takiyuddin et al. (15), indicating that peak chromogranin A concentrations may occur earlier than 15 min postexercise. The increase in chromogranin A concentrations after exercise in our study was modest, reaching values less than those previously reported in patients with essential hypertensive (5) and considerably less than the plasma concentrations seen in patients with proven pheochromocytomas who have plasma concentrations generally >100 μg/L and often >1000 μg/L (6). Because strenuous exercise did not increase plasma chromogranin A concentrations into the range seen in patients with pheochromocytoma, restrictions on physical activity before measuring chromogranin A in hypertensive patients with suspected pheochromocytoma may be unnecessary, and physical activity before such measurements should not obscure the diagnostic utility of plasma chromogranin A measurement in the screening process.

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References


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REMED1 Drug Profiling System Readily Distinguishable between Cyclobenzaprine and Amitriptyline in Emergency Toxicology Urine Specimens

To the Editor:

Cyclobenzaprine (Flexeril) is a skeletal muscle relaxant indicated for relief of spasms associated with acute skeletal muscle pain. Structurally, cyclobenzaprine differs from amitriptyline only by a double bond in the central cycloheptyl ring; therefore, the drug is often difficult to distinguish from amitriptyline by methods used in emergency toxicology testing. Cyclobenzaprine cross-reacts in the EMIT and Abbott ADx tricyclic antidepressant immunoassays (1, 2). It has retention times similar to amitriptyline in popular gas-liquid-chromatographic and high-pressure liquid-chromatographic (HPLC) systems and is difficult to resolve from amitriptyline in classical thin-layer chromatographic systems (3). Cyclobenzaprine has staining characteristics in the TOXILAB commercial thin-layer chromatography system different from those of amitriptyline. In stage III, TOXILAB A, amitriptyline fluoresces pink, whereas cyclobenzaprine gives an orange color. However, the intensity of the fluorescence is concentration-dependent and identification may be questionable.

The extra double bond in cyclobenzaprine produces an ultraviolet spectrum readily distinguished from amitriptyline or other common tricyclic antidepressants. Cyclobenzaprine has a maximum absorbance at 290 nm in aqueous acid with no alkaline shift. Amitriptyline has a maximum absorbance at 239 nm in aqueous acid. HPLC with photodiode array detection readily distinguishes between cy-