Evaluation of Circulating Galanin Levels After Exercise-Induced Pituitary Hormone Secretion in Man

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The neuropeptide galanin (GAL) is widely distributed in the central and peripheral nervous systems, anterior pituitary, and adrenal medulla. GAL is colocalized with corticotropin (ACTH) in the human pituitary and with epinephrine (E) and norepinephrine (NE) in chromaffin cells of the adrenal medulla. The function of GAL in peripheral tissues is not known, although the presence of the peptide in corticotrophs and the adrenal gland suggest that it participates in stress responses. In the present study, we investigated whether GAL is cosecreted with ACTH during activation of corticotrophs by an acute physical exercise test. Circulating levels of GAL and pituitary hormones were measured in healthy exercise-tested and control male subjects. Blood samples were collected during basal conditions, maximal power output (MPO), and the recovery period. Control subjects were sampled during the resting condition. The pituitary response to exercise was characterized by a significant increase in ACTH plasma levels (peak value 13.28 ± 2.19 *v* 6.68 ± 1.01 pmol/L, $P < .05$) and growth hormone (GH) serum levels (peak value, 14.53 ± 5.59 *v* 0.29 ± 0.1 μg/L, $P < .02$), with the peak in hormone levels detected 15 minutes after the end of exercise. No change in circulating prolactin (PRL) levels was detected. An expected significant increase in plasma levels of both E (peak value, 1,574.41 ± 403.31 *v* 267.44 ± 60.03 pmol/L, $P < .01$) and NE (peak value, 7,275.25 ± 955.80 *v* 961.51 ± 168.40 pmol/L, $P < .01$) was also observed. Plasma GAL levels were not affected by the acute exercise test, with the levels being comparable to baseline during the exercise test and the recovery phase. At any sample time, GAL values were comparable between exercise-tested and control subjects. These data show that despite the colocalization of GAL and ACTH within the same pituitary cells, the two peptides are not coreleased in response to stress resulting from acute physical exercise. Furthermore, pituitary GAL seems not to be involved in the stimulation of GH secretion in exercise-tested subjects. The results also indicate that GAL is not coreleased with E or NE in response to the exercise-induced stress condition.

GALANIN (GAL) is a 29 (30 in humans)–amino acid peptide initially isolated from porcine intestine and subsequently found to be widely distributed in the central and peripheral nervous systems, respiratory and genitourinary tracts, anterior pituitary gland, and adrenal medulla.[1-4] Since its discovery, numerous studies have suggested that GAL may be involved in anterior pituitary hormone secretion. In rats, GAL modulates prolactin (PRL), gonadotropin, and growth hormone (GH) secretion.[5,6] In humans, GAL primarily affects GH secretion.[7,8] In addition, an increase of the PRL response to an arginine challenge following GAL administration has also been reported in human subjects.[9] Within the rodent pituitary, GAL is located in lactotrophs, thyrotrophs, and somatotrophs.[10] In the human pituitary, the location of GAL is more restricted, with GAL-like immunoreactivity[11] or GAL mRNA[12] being localized in the corticotrophs of healthy or adenomatous pituitaries.[11] These observations suggest a close relationship between corticotropin (ACTH) and GAL secretion.[12]

In addition to the possible role for GAL in the regulation of anterior pituitary hormone secretion, these findings suggest that GAL, produced in the anterior pituitary, may be secreted into the peripheral circulation along with other pituitary hormones. It is well known that certain stressful stimuli lead to an increase of secretion of GH,[13] PRL,[14] and ACTH.[15] To ascertain whether GAL can be cosecreted with ACTH from corticotrophs in response to stressful stimuli, the present study evaluated plasma GAL and ACTH levels and GH and PRL serum levels during an acute physical exercise test in human subjects. Moreover, since GAL has been detected in both neuronal structures and adrenomedullary cells of the sympathoadrenal system,[2,3] we also evaluated concomitant changes in plasma levels of catecholamines (CATs) in our subjects.

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SUBJECTS AND METHODS

Subjects

Eight men aged 24.9 ± 3.2 years with a body mass index of 25.9 ± 0.3% were studied. The subjects were enrolled after a complete clinical and laboratory examination including electrocardiographic evaluation to exclude individuals with any pathological condition. The subjects were free of any medication, two had a low tobacco intake (three to five cigarettes per day), and one of them also had a low caffeine intake; two other subjects had a low alcohol intake (<4 g/d). Seven healthy subjects matched for age, sex, and body mass index were used as a control group. Caffeine, alcohol, and tobacco intakes in these subjects were comparable to those of the tested group. Subjects were asked to refrain from smoking for a 10-hour period preceding the study. All subjects provided informed consent to participate in the study, and the protocol was approved by the local Ethics Committee of the University of Parma.

Acute Physical Exercise Test

The study started at 8 AM after an overnight fast. A 19-gauge cannula was inserted into a forearm vein, and the needle was kept patent throughout the experiment by a slow saline infusion. The subjects performed physical exercise on an electromagnetically braked cycloergometer (Siemens Elema M84, Solna, Sweden).

Since a direct correlation between the maximal work capacity, expressed as oxygen consumption observed during physical exercise, and the increase in heart rate (HR) has been reported,[16,17] we evaluated maximal work capacity by calculating the maximal predicted HR (MPHR) based on the age of the subject.[18] The workload was gradually increased by 30 W every 3 minutes to reach a HR greater than 90% of the MPHR, corresponding to what we considered the maximal power output (MPO). At that point, the exercise was stopped and the subjects were allowed to rest for 30 minutes. Room temperature was maintained

at 21°C. The interruption criteria were physical exhaustion or attainment of the MPHR.

The systolic blood pressure (SBP), HR, and electrocardiogram were evaluated continuously during the study. Blood samples were collected 30 minutes before the onset of exercise (time −30), at time 0, and subsequently when the HR was at least 50% of the MPHR and during MPO. Blood was also taken 15 and 30 minutes after termination of exercise. Blood was collected in chilled tubes containing EDTA (1 mg/mL blood) and aprotinin (500 KIU/mL blood) for plasma ACTH and GAL evaluation, and in heparinized chilled tubes for epinephrine (E) and norepinephrine (NE) determination. Immediately after withdrawal, blood was centrifuged at 4°C for plasma collection. Samples for CAT assay were kept in dark tubes until the plasma was separated. Afterward, plasma was stored at −80°C. Blood for serum GH and PRL evaluation was also collected at the same time points already described. Serum was stored at −20°C until hormonal evaluation.

Control subjects underwent sampling in the resting condition over the same period. Since in the exercising group, time points corresponding to 50% of the MPHR and MPO occurred after 5 to 7 minutes and 12 to 16 minutes from the beginning of exercise, the sampling relating to these two time points in control subjects was performed at 6 and 14 minutes, respectively, from time 0.

Evaluation of Circulating GAL, CAT, and Hormonal Levels

Plasma samples for GAL evaluation were extracted in 1% trifluoroacetic acid and 60% acetonitrile using C18 reverse-phase cartridges (Sep-columns; Peninsula Laboratories, Belmont, CA). After extraction, the samples were dried in a vacuum centrifuge and resuspended in phosphate buffer. All reagents were purchased from Peninsula Laboratories. Measurements of GAL levels were performed by radioimmunoassay (RIA) using kits also from Peninsula Laboratories. The rabbit anti–human GAL antiserum used in our GAL RIA was shown to have 100% cross-reactivity with both rat and porcine GALs, whereas no cross-reactivity with porcine GAL message-associated peptide, substance P, human vasointestinal polypeptide, and human secretin was documented (data provided by Peninsula Laboratories).

Circulating levels of ACTH were determined in unextracted plasma by an immunoradiometric assay using a commercial kit (Nichols, San Juan Capistrano, CA) with monoclonal antibodies directed at the ACTH 1-17 sequence. Serum GH levels were measured by an immunoradiometric assay using a commercially available kit (Sorin, Milan, Italy). Serum levels of PRL were measured by an enzyme-linked immunoassay using monoclonal antibodies (Ares-Serono, Milan, Italy). Circulating levels of E and NE were evaluated within 2 weeks of blood collection by high-performance liquid chromatography using an electrochemical detector[19] after plasma extraction.[20]

Sensitivities of the methods were 27.25 pmol/L and 29.55 pmol/L for E and NE, respectively, and 0.3 pmol/L, 0.15 μg/L, and 0.2 μg/L for ACTH, GH, and PRL, respectively. The GAL RIA had a sensitivity of 0.77 pmol/L, with an ED_{50} of 34.87 pmol/L and an IC_{80} of 9.3 pmol/L. All measurements were performed in duplicate. Intraassay and interassay coefficients of variation for the GAL assay were 11.2% and 10.8%, respectively, at 1.58 pmol/L, and 10.9% and 11.7%, respectively, at 22.16 pmol/L. For all other parameters, interassay and intraassay variabilities were less than 10%.

Statistical Analysis

The Statistical Analysis System software program (SAS Institute, Cary, NC) was used for all data analyses. A preliminary analysis showed that the data sets did not conform to a normal distribution. For this reason, all values were logarithmically transformed before statistical calculations. The curve responses for each parameter were then analyzed using ANOVA followed by an adjusted *t* test to detect differences between means. Data are expressed as the mean ± SEM.

Table 1. HR and SBP in Exercise-Tested Subjects at Baseline and During MPO

	Baseline	MPO	*P*
HR (beats/min)	77.14 ± 3.50	180.17 ± 1.78	<.001
SBP (mm Hg)	117.08 ± 2.78	194.58 ± 3.96	<.001

RESULTS

The exercise test produced an increase in SBP and HR ($P < .001$) in all subjects, with maximum values concomitant with the MPO (Table 1).

Basal ACTH levels were comparable between tested and control subjects. Exercise resulted in a moderate but significant ($P < .05$) increase in ACTH levels (Fig 1), which increased from a basal value of 6.68 ± 1.01 pmol/L to a peak of 13.28 ± 2.19 pmol/L at 15 minutes from the end of exercise; ACTH levels returned to values close to the baseline at 30 minutes from the end of exercise. No significant changes in ACTH plasma levels over baseline were documented in control subjects. Plasma GAL levels were found to be nonsignificantly affected by the exercise test; at any sample time, GAL values were comparable between control and exercising subjects and were not significantly different from baseline (Fig 2). The power of the test was calculated by methods described by Pearson and Hartley[21] for an α value of .05, and the value was .53 and .78 for increases in GAL levels of 50% and 100%, respectively, above baseline.

Serum GH levels increased significantly in exercise-tested subjects, with peak values of 14.53 ± 5.59 μg/L versus basal values of 0.29 ± 0.1 μg/L ($P < .02$), although a high variability in the responses was observed (data not shown). GH levels increased significantly from the time point corresponding to MPO and reached peak values 15 minutes after the end of exercise. GH values were still significantly ($P < .05$) elevated above baseline 30 minutes after the end of the exercise test.

Basal PRL levels were comparable between the two groups. No significant changes in PRL levels were observed throughout

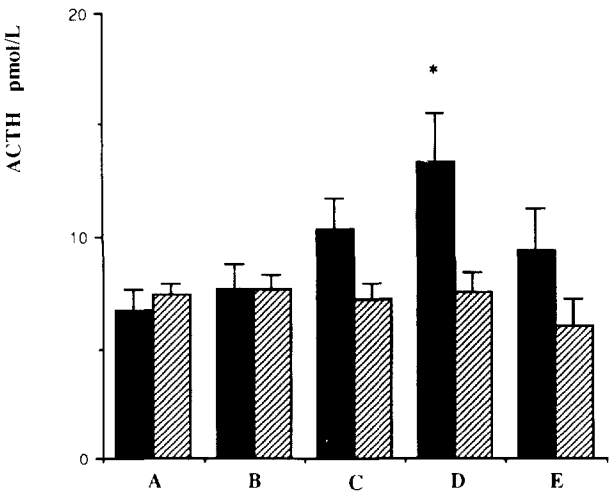

Fig 1. Plasma ACTH levels in exercise-tested subjects (■) and controls (▨). (A) Mean of 2 −30 and 0 minutes basal values; (B) 50% of the MPHR; (C) MPO; (D) +15 minutes from the end of exercise; (E) +30 minutes from the end of exercise. *$P < .05$ *v* baseline.

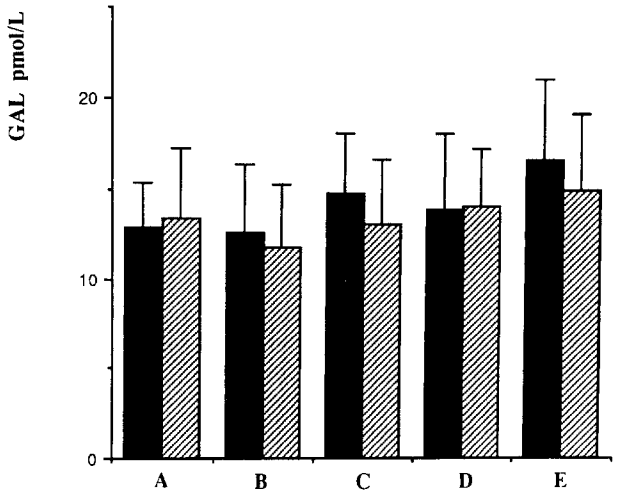

Fig 2. Plasma GAL levels in exercise-tested subjects (■) and controls (▨). See Fig 1 for details.

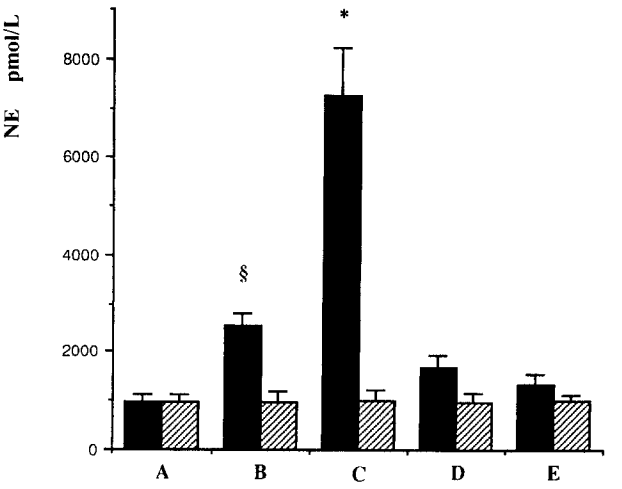

Fig 4. Plasma NE levels in exercise-tested subjects (■) and controls (▨). *$P < .01$ *v* baseline. §$P < .05$ *v* baseline. See Fig 1 for details.

the study either in tested or in control subjects (data not shown). The power of the test for evaluation of PRL levels was calculated[21] for an α value of .05, and the value was .88 and greater than .99 for increases in PRL values of 50% and 100%, respectively, above baseline.

Levels of E and NE in plasma of exercising subjects (Figs 3 and 4) were significantly ($P < .01$) elevated above baseline. E increased to a peak of 1,574.41 ± 403.31 pmol/L from the basal value of 267.44 ± 60.03 pmol/L; NE increased from basal value of 961.51 ± 168.40 pmol/L to a peak of 7,275.25 ± 955.80 pmol/L. For both CAT levels, the peak corresponded to MPO. In addition, a significant ($P < .05$) increase in NE levels over baseline was found in samples collected at the time point corresponding to 50% of the MPHR. In samples collected 15 minutes after the end of exercise, both E and NE levels returned to baseline without subsequent changes. No significant modifications of either E or NE levels over baseline were detected in control subjects during the whole period of the study (Figs 3 and 4).

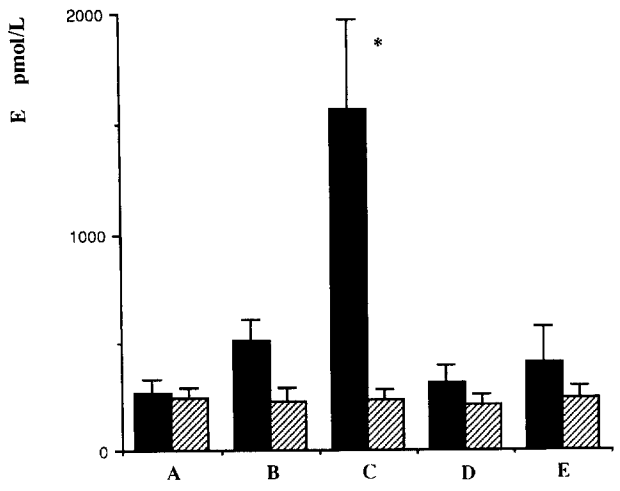

Fig 3. Plasma E levels in exercise-tested subjects (■) and controls (▨). *$P < .01$ *v* baseline. See Fig 1 for details.

DISCUSSION

The present results demonstrate that in our experimental conditions, exercise results in activation of the sympathoadrenal system and release of anterior pituitary hormones, both characteristic of a stress response. The present findings that ACTH plasma levels increased during acute physical exercise are in agreement with previous reports.[15] Since GAL is colocalized with ACTH in the anterior pituitary, the present experiments were designed to determine whether GAL is cosecreted with ACTH under physiological stimulation of corticotrophs by physical exercise. Our observations clearly show that elevated levels of ACTH in exercised subjects were not accompanied by increased GAL levels in peripheral blood. These results therefore suggest that GAL is not coreleased with ACTH from human corticotrophs, at least under physiological stimuli such as exercising. The increase in ACTH plasma levels in exercising subjects was moderate but significant, and one could therefore hypothesize that stimulation by physical exercise was sufficient to elicit an elevation in ACTH secretion but insufficient to increase GAL secretion from corticotrophs. ACTH-releasing hormone and other hypothalamic peptides (ie, vasopressin) are thought to be the most important factors responsible for ACTH release from corticotrophs during physical exercise.[22] In this regard, our data suggest the possibility that the control of GAL release from corticotrophs is less sensitive to hypothalamic ACTH-releasing factor or vasopressin than ACTH. Alternatively, the possibility that GAL is not secreted from corticotrophs under classic physiological hypothalamic stimuli cannot be ruled out.

In agreement with previous reports,[13] the present study shows an increase of GH circulating levels during the exercise test, with a peak occurring 15 minutes after the end of the stimulus. GAL has been shown to increase circulating GH levels when administered exogenously in rats and humans,[6-9] possibly acting at the hypothalamic level.[6] However, the present study did not

show any significant increase in plasma GAL levels concomitant with the increments in circulating GH levels, suggesting that circulating GAL is not involved in the physiological GH response to physical exercise.

In agreement with a previous report,[23] we did not find significant changes in PRL levels during the whole period of observation. Since no changes in either PRL or GAL were found, we are not able to speculate on the possible relationship between PRL release and circulating GAL levels in man.

Studies in rats have shown that GAL is secreted from the pituitary gland into the bloodstream under basal conditions and that pituitary GAL contributes approximately 30% of the circulating peptide.[24] Furthermore, the pituitary secretes GAL in response to certain stimuli in rats.[25] Based on these reports, we expected an increase in GAL circulating levels in our study; however, our data clearly show a lack of response of plasma GAL in physical stress.

When CAT levels were evaluated in our tested subjects, clear-cut increments were found for both E and NE levels, with a peak for both CATs concomitant with MPO. Plasma NE has been demonstrated to be primarily derived from nerve terminals under basal conditions, whereas following stressful stimuli a significant proportion of NE is secreted from the adrenal medulla.[26] Circulating E levels are mainly regulated by secretory phenomena from adrenomedullary cells.[27] Since GAL has been shown to be colocalized with CATs in chromaffin cells of the adrenal medulla[16,28] and to be released in in vitro models following certain stimuli,[29] an increase in GAL levels concomitant with CAT increases could be expected in our experiment. However, our data failed to demonstrate any significant increase in GAL levels during both the test and the recovery period. The present findings are in agreement with results from a previous study documenting the lack of effect of physical exercise on circulating GAL levels.[30] However, in the study reported by Sundkvist et al,[30] circulating levels of pituitary hormones were not measured, and therefore, the relationship between exercise-induced pituitary hormone secretion and peripheral GAL levels could not be evaluated. Moreover, in the present study, values for circulating GAL in human blood were measured using an antiserum directed against GAL, with a higher sensitivity than the antiserum against porcine GAL used in the previous study.

Taken together, these data demonstrate that despite the colocalization of GAL and ACTH within the same pituitary cells, the two peptides are not coreleased in response to physiological stimulation by acute physical exercise. Furthermore, circulating GAL levels seem not to be involved in the increase of GH secretion seen in exercise-tested subjects. Finally, our results further indicate that GAL is not coreleased with either E or NE from the adrenal medulla in response to the exercise-induced stress condition.

The present observations leave unsolved the question of the meaning of circulating GAL in human physiology. However, it could be hypothesized that in humans the peptide acts locally in autocrine or paracrine fashion to modulate the secretion of pituitary hormones or CATs. In this view, circulating levels of GAL would merely represent the result of a leakage phenomenon from the sites of synthesis, rather than a regulated secretory event. However, basal GAL levels are on the same order of magnitude of substances known to be secreted into the peripheral circulation to reach specific targets (ie, ACTH), suggesting that a mere leakage phenomenon is unlikely to be the only source of circulating GAL. More detailed studies are needed to better address the question of the origin and physiological role of circulating GAL levels in humans.

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