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Treadmill exercise-induced stress causes a rise of blood histamine in normotensive but not in primary hypertensive humans

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Abstract

We have previously shown an interaction between noradrenergic and histamine-containing neurons in the rat vas deferens. As a generalized phenomenon, this interaction is involved in a novel peripheral reflex that, in an inhibitory way, modulates sympathetic activity and arterial pressure. Consistent with this, an activation of postganglionic sympathetic neurons causes a rise in rat blood histamine. In the present study, we showed that enhanced sympathetic activity due to treadmill exercise in normotensive humans, is accompanied by a rise in blood histamine, suggesting the presence of a similar neuronal interaction in humans. In contrast, the rise in blood histamine does not occur in primary hypertensive humans during the same degree of physical exercise, suggesting that this interaction is faulty in such hypertensives and could be involved in the pathophysiology of the disease.

Keywords: Peripheral reflex; Sympathetic system; Neuronal histamine; Blood; Noradrenergic neuron; Stress; Hypertension

1. Introduction

The discovery of a peripheral reflex that down regulates sympathetic activity in the rat vas deferens (Campos, 1983, 1988; Campos and Briceño, 1992) prompted the search for similar reflexes in other species and organs. A similar inhibitory modulation of peripheral sympathetic activity was found in the dog heart (Campos and Briceño, 1992). An interaction between noradrenergic and histamine-containing neurons appears to be involved in this peripheral short-loop reflex (Campos and Dominguez, 1995), in which neuronal histamine could be modulating sympathetic activity in an inhibitory way. Thus, surgical interruptions of histamine-containing neuronal pathways adjacent to the sympathetic nerves of the rat vas deferens cause local facilitation of sympathetic activity (Campos and Briceño, 1992). In addition, irreversible inhibition of neuronal histamine biosynthesis with α-fluoromethylhistidine causes an overall facilitation of sympathetic activity and arterial hypertension in the rat (Domínguez et al., 1991; Campos et al., 1996) suggesting the existence of a generalized mechanism of sympathetic autoregulation. Furthermore, enhanced sympathetic activity due to footshock stress in the rat is accompanied by a rise in blood histamine, which is dependent upon the activation of postganglionic sympathetic neurons (Campos and Montenegro, 1998). In other words, neuronal histamine appears to be reflexly released, as an overall compensatory phenomenon, during enhanced sympathetic activity. Histamine may be inhibiting noradrenaline release from sympathetic nerve terminals, and thus vascular responses due to stress, via H_3 inhibitory receptors (Acuña et al., 1998). In the context of the findings described above, we now report on the effect of treadmill exercise-induced stress on blood histamine levels in normotensive and primary hypertensive humans.

2. Materials and methods

2.1. Subjects

Twenty-six human males, aged from 24 to 68 years (42 ± 10, mean $\pm$ S.D.), were studied. An informed consent was obtained from every subject. The control group

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($N = 17$) was made up of healthy normotensive volunteers, without any previous record of chronic disease, including obesity, diabetes or allergy. The hypertensive group ($N = 9$) was made up of patients diagnosed with primary hypertension (untreated supine diastolic blood pressure > 100 mm Hg), with no signs of endocrine, renal, gastrointestinal or nervous system diseases. Patients with a history of drug or alcohol abuse, grade 3 or 4 retinopathy, hepatic or hematological disorders, were excluded. The hypertensive group was under placebo treatment for at least 1 week (two patients for 2 weeks; three had never received antihypertensive medication because they were not aware of their illness) prior to the start of the study. Both groups had fasted for at least 12 h before the start of measurements, always performed in the morning. Electrocardiogram recording was monitored throughout the study period, and heart rate was determined from lead II. Arterial pressure was measured with a mercury sphygmomanometer. Mean arterial pressure was calculated from diastolic + 1/3 (systolic − diastolic pressure).

2.2. Blood sampling and fluorometric analysis of histamine

A venous scalp No. 19 and a catheter were inserted in the antecubital vein for collection of 5-ml blood samples at the end of each experimental step. The catheter was filled with heparinized saline (200 U/ml) to avoid clotting. The dead space was approximately 1 ml. Two milliliters of blood partially diluted with saline was discarded before each sample collection for fluorometric determination of histamine according to Schwartz et al. (1970) with modifications at the elution step from the cellulose column (Campos and Montenegro, 1998). Blood samples were placed in chilled plastic tubes containing 0.1 ml 5% sodium EDTA in saline and mixed thoroughly. Proteins were precipitated by the addition of 5 ml 1 N $HCLO_4$ and centrifugation in the cold at 12,000 rpm for 15 min. During the isolation of histamine, catecholamines (nor- or adrenaline, 1 mg each) or heparin (100 U) added to samples or blanks did not interfere with the histamine determination. A priming 1.5 ml 0.4 M NaCl aliquot was added to the cellulose column and discarded. Then, elution was performed with 2 ml 0.2 M NaCl, which was collected for fluorophore development. No histamine was lost from the column with the priming aliquot as checked with tritiated histamine or fluorescence reading. Recovery of histamine added to blood samples (50–400 ng) was over 90%.

2.3. Active uptake of histamine in human blood cells

An additional group of five healthy subjects was included as blood donors for the study of histamine uptake in human blood cells in vitro. A 20-ml blood sample was drawn from each subject. The sample was divided into four 5-ml aliquots. Half of these were incubated at 37°C, and the other half at 4°C in a metabolic shaker. A 5-min equilibration period was allowed before the addition of 5 μg histamine in 100 μl saline to every sample . Incubation proceeded for an additional 5-min period. At the end of this period, all samples were placed in ice-cold water. One sample incubated at one of the two temperatures was centrifuged in the cold at 7500 rpm for 5 min to obtain plasma and cellular elements. The latter were washed once with 3 ml 0.1 M sodium phosphate buffer, pH 7.0. To precipitate proteins, 5 ml 1 N $HCLO_4$ was added to sediments and whole blood samples, while 3 ml 1 N $HCLO_4$ was added to plasma. After the addition of acid, centrifugation in the cold was performed at 12,000 rpm for 15 min. The plasma supernatant was pooled with the corresponding sediment wash.

2.4. Experimental protocol

The experimental protocol was as follows: (1) insertion of a scalp in the antecubital vein; 20-min rest in the supine position in a quiet air-conditioned room, at approximately 22°C. (2) 5-min standing; (3) physical exercise on a treadmill (Quinton Instruments) according to the protocol of a multistage method (Bruce and Hornsten, 1969). (4) 20-min rest in the supine position. Blood samples were drawn at the end of resting or standing and during the last 15 s of each 3-min stage of exercise.

2.5. Statistics

Student's *t*-test for paired samples was used for active uptake of histamine in vitro. Repeated Measures Analysis

Table 1
Active uptake of histamine in human blood cells
$n = 5$, 3 men and 2 women. Values are expressed in μg per sample, mean ± S.E.M. Active uptake was obtained by subtracting values for histamine levels in cells at 4°C from values at 37°C. Incubations were for 5 min.

Cells	Plasma	Total	Whole blood	Cellular active uptake
4°C				
0.144 ± 0.030	4.305 ± 0.191	4.450 ± 0.213	4.257 ± 0.200	
37°C				
0.558 ± 0.046[a]	3.910 ± 0.273	4.468 ± 0.277	4.393 ± 0.299	0.414 ± 0.058

[a] $P < 0.002$.

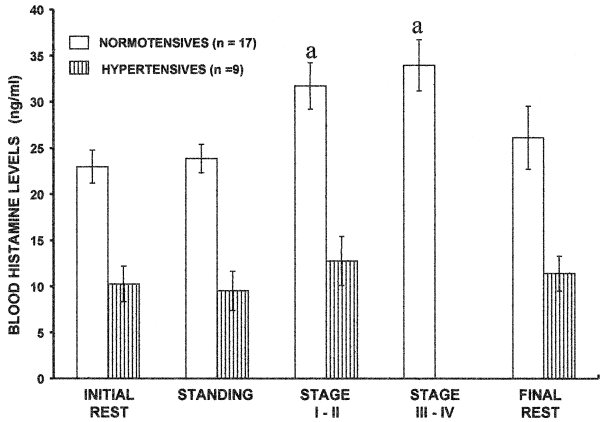

Fig. 1. Changes in blood histamine in normotensive and primary hypertensive humans after treadmill exercise-induced stress. A multistage method was used according to Bruce and Hornsten (1969). Bars represent mean ± S.E.M. of number of study subjects indicated in parenthesis. [a]Vs Initial rest, $P < 0.01$.

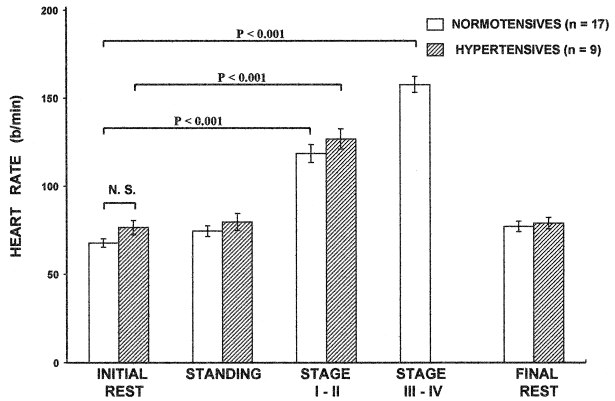

Fig. 3. Changes in heart rate in normotensive and primary hypertensive humans after treadmill exercise-induced stress. For further details, see Fig. 1.

of Variance and the Tuckey–Kramer Multiple Comparisons Test were used for evaluating differences in histamine levels in blood samples in vivo. $P < 0.05$ was considered significant.

3. Results

3.1. Active uptake of histamine in human blood cells

Histamine was actively taken up into human blood cellular elements when whole blood was incubated with the amine for 5 min at 37°C (Table 1).

3.2. Changes in human blood histamine levels during treadmill exercise in normotensive and hypertensives subjects

Blood histamine levels at rest (supine position) in hypertensives were almost half the corresponding values of the normotensives (Fig. 1). When the subjects moved from the supine to the standing position, there was no change in blood histamine levels in comparison to initial rest values in either group. Under the conditions of our study, during physical exercise, there was an enhancement of sympathetic activity in both groups of subjects as judged from the increase in mean arterial pressure and heart rate (Figs. 2 and 3). Normotensive subjects showed a rise in blood histamine levels from the beginning of exercise, some at stage I and others at stage II. Therefore, the greater rise at the stage I–II interval is presented. For the same reason, data from the stage III to IV interval are also presented. Blood histamine levels approached the initial rest levels after the final rest period. Hypertensives showed no change in blood histamine levels at stage I–II of the Bruce protocol in comparison to the initial rest levels (Fig. 1). For obvious reasons, the hypertensives were allowed to reach only stage II of the exercise test, which represents a mild effort with no harmful effects.

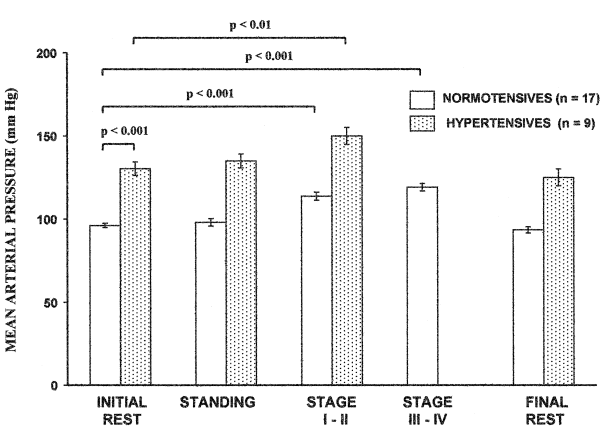

Fig. 2. Changes in mean arterial pressure in normotensive and primary hypertensive humans after treadmill exercise-induced stress. For further details, see Fig. 1.

4. Discussion

We measured histamine levels in whole human blood since, as in the rat and other mammals (Anrep et al., 1939; Johnson et al., 1966), histamine may be taken up into blood cellular elements, as shown in vitro with human blood in the present work (Table 1). This could explain why pronounced increases in blood histamine during an asthmatic crisis are detected in whole blood but not in plasma (Izumi et al., 1984). Therefore, it was safer to measure rapid changes of histamine levels in whole blood instead of plasma during increased sympathetic activity.

Under conditions of increased sympathetic activity, as judged from the significant increases in mean arterial pressure and heart rate during physical exercise in both groups of study subjects (Figs. 2 and 3), treadmill exercise

causes an increase in blood histamine levels at I–II and sustained increases at III–IV stages of the Bruce protocol in normotensive subjects as compared to initial rest values (Fig 1). In contrast, stage I–II of the exercise test does not cause a rise in blood histamine in hypertensives, as compared to initial rest values.

The rise in blood histamine levels in normotensives occurs with relatively short exercise intervals (3–6 min) and, according to its extent, it appears not to be due to hemoconcentration. It seems unlikely that mobilisation of a histamine pool sequestered at the periphery is responsible for the histamine rise when cardiovascular responses are enhanced during exercise, since histamine rises do not occur in primary hypertensives in the presence of enhanced cardiovascular responses due to exercise.

We have previously shown that 5-min footshock stress causes a rise in rat blood histamine, which is dependent on the activation of postganglionic sympathetic neurons (Campos and Montenegro, 1998). Histamine appears to be released as a compensatory phenomenon when sympathetic activity is increased. The released histamine, which might be inhibiting sympathetic activity, seems to be neuronal in origin as short-term (4 h) irreversible inhibition of histamine biosynthesis causes presynaptic facilitation of sympathetic activity (Campos et al., 1996). In the same direction, local neuronal histamine depletion through nerve degeneration causes local facilitation of vas deferens sympathetic activity (Campos and Briceño, 1992). Histamine inhibitory modulation of sympathetic activity seems to occur through histamine H_3 receptors located at sympathetic nerve terminals as shown for vasopressor responses to footshock stress (Acuña et al., 1998).

The rise in blood histamine in humans after short-term (3–6 min) treadmill exercise-induced stress is perhaps also involved in the inhibitory modulation of sympathetic activity. This type of stress causes a rise in blood noradrenaline (Galbo et al., 1975), and exercise with a bicycle ergometer increases both blood noradrenaline (Häggendal et al., 1970; Pernow et al., 1986) and histamine (Dunner and Pernow, 1958). However, these findings do not prove a causal relationship between the increases in both amines.

Non-mast cell histamine has been identified in the human vascular wall (Brody, 1980) and histamine H_3 inhibitory receptors have been shown to be present in human heart sympathetic nerve terminals (Imamura et al., 1995) and saphenous vein (Molderings et al., 1991), but still the evidence is meager concerning the possible existence of a mechanism of this sort inhibiting sympathetic activity in humans. On the other hand, the anticholinesterase edrophonium, which is known to stimulate indirectly postganglionic sympathetic neurons in humans, as revealed by a rise in blood noradrenaline (Leveston et al., 1979), causes a similar rise in blood histamine. However, the edrophonium-induced rise in blood histamine is blunted in diabetic patients suffering from peripheral neuropathy and orthostatic hypotension (unpublished observations) as is the rise in blood noradrenaline (Leveston et al., 1979). These findings suggest that, under normal conditions, the edrophonium-induced peripheral sympathetic discharge is causing the rise in human blood histamine as does the sympathetic discharge in the rat submitted to footshock stress (Campos and Montenegro, 1998). A similar interaction could occur when sympathetic activity is enhanced during the treadmill exercise-induced stress that results in a rise in blood histamine.

In addition, histamine blood levels at rest in primary hypertensives are about half the baseline levels of the normotensives under the same conditions (Fig. 1), which suggests that, at some step of the formation, storage, catabolism and/or release of histamine into the circulation, the mechanism is altered in primary hypertensives as compared to that in normotensives. This suggestion gains support from the finding that treadmill exercise-induced stress does not cause a rise in blood histamine levels in primary hypertensives as it does in normotensives.

The present findings of reduced resting levels of blood histamine in primary hypertensives, together with the failure of blood histamine to rise in these subjects during exercise-induced stress relative to those in normotensives, should be considered in further studies related to the pathophysiology of primary arterial hypertension.

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