Influence of voluntary exercise on hypothalamic norepinephrine

**Lambert, Gavin W., and Ingibjorg H. Jonsdottir.** Influence of voluntary exercise on hypothalamic norepinephrine. *J. Appl. Physiol.* 85(3): 962–966, 1998.—We combined hypothalamic tissue and plasma determinations of norepinephrine, dihydroxyphenylalanine, and dihydroxyphenylglycol with measurements of abdominal fat in voluntary running rats to examine the relationship among exercise training, hypothalamic and sympathetic nervous function, and body fat stores. The hypothalamic concentrations of norepinephrine, dihydroxyphenylalanine, and dihydroxyphenylglycol were reduced after exercise training (P < 0.01), with the amount of norepinephrine being strongly associated with the plasma norepinephrine (r = 0.58, P < 0.05) and dihydroxyphenylglycol (r = 0.65, P < 0.01) concentrations. Exercise training resulted in a diminution in abdominal fat mass (P < 0.01). A strong relationship existed between fat mass and hypothalamic norepinephrine content (r = 0.83, P < 0.001). The presence of a positive relationship between the arterial and hypothalamic norepinephrine levels provides presumptive evidence of an association between noradrenergic neuronal activity of the hypothalamus and sympathetic nervous function. The observation that abdominal fat mass is linked with norepinephrine in the hypothalamus raises the possibility that alterations in body fat stores provide an afferent signal linking hypothalamic function and the activity of the sympathetic nervous system.

The virtues of regular aerobic exercise have been extolled on the basis that exercise regimes are associated with a diminution in systemic blood pressure in hypertensive patients (25), with a reduction in rates of cardiovascular mortality (26) and with a concomitant lowering of body fat and obese gene expression in animal experiments (12, 38). Although the precise mechanism underlying these effects is debatable, a consistent finding among many studies is that regular exercise training is associated with a reduction in overall sympathetic nervous activity (5, 18, 24, 25, 34).

Given that previous reports conducted in human subjects have illustrated some dependence of sympathetic outflow on cerebral noradrenergic activity (9, 10), one could speculate that some of the beneficial effects of exercise training are mediated via a modulation of brain noradrenergic processes. By virtue of its extensive neuronal circuitry projecting to autonomic premotor nuclei such as the A5 noradrenergic cell group and the rostral ventrolateral medulla (13), the hypothalamus may hold a pivotal role in the regulation of autonomic reflexes. Knowledge that the obese gene product leptin acts, purportedly via the paraventricular nucleus of the hypothalamus (36), to increase energy expenditure and reduce body fat stores raises the possibility that the alterations in fat mass that occur after repeated bouts of exercise may, at least in part, provide the afferent signal to invoke alterations in hypothalamic function.

With this in mind we sought to examine the modulatory effect that repeated voluntary exercise exerts on noradrenergic function of the hypothalamus and body fat stores. By using the spontaneously running rat model established in our department (31), we examined the influence of both running distance and duration on the hypothalamic content of norepinephrine, its precursor dihydroxyphenylalanine (DOPA) and its intraneuronal metabolite dihydroxyphenylglycol (DHPG), and on abdominal fat mass. Although acknowledging that plasma levels of norepinephrine, on a hierarchical scale of biochemical indicators of global sympathetic nervous activation, lag behind the resolution achieved with isotope-dilution techniques (7, 8), we used the arterial plasma norepinephrine level as a surrogate indicator of sympathetic nervous function to examine the possible interplay among exercise training, the hypothalamus, and the sympathetic nervous system.

**MATERIALS AND METHODS**

Data obtained from 20 male spontaneously hypertensive rats (Møllegaards Breeding Centre) form the basis of this report. On arrival at our center, the 8-wk-old rats were randomly assigned into three groups: control and 6 and 11 wk of exercise training. Animals were housed in individual cages of identical dimensions (40 × 24 × 15 cm) for the duration of the experiment and were kept in a constant 12:12-h light-dark cycle (light 0700–1900 h) in a room maintained at 24°C with a relative humidity of 50–60% and with free access to standard rat chow and water. The cages of the exercising animals were modified by the attachment of a wheel of 22.5 cm diameter to which the animals had free access. Wheel revolutions were automatically registered by a microprocessor and data were printed out every 30 min. The running apparatus and the voluntary running behavior of the spontaneously hypertensive rat have been described in detail in an earlier report by Shyu et al. (31). In our experience, the spontaneously hypertensive rat exhibits a more pronounced and reproducible capacity to exercise voluntarily when exposed to a running wheel than do animals of the Sprague-Dawley or Wistar-Kyoto strains. Studies from our group have previously documented the effectiveness of 5–6 wk of exercise training in improving cardiovascular performance and immune function in the voluntary running, spontaneously hypertensive rat (11, 19).

All running animals were permitted to run voluntarily in their wheels for either 6 or 11 wk. Forty-eight hours before the final day of exercise, animals were anesthetized with methohexital sodium (50 mg/kg body wt ip), the jugular vein was cannulated, and a catheter was tunneled under the skin and exteriorized via a skin incision in the neck for subsequent blood sampling. The animals were allowed to recover from the anesthesia before being killed by decapitation.
procedure and were then returned to their cages. Twenty-four hours later (i.e., 24 h before experimentation), the running wheel was locked. On the following day, 1.5-ml blood samples were obtained via the jugular vein from conscious, freely moving animals. Because of technical difficulties, blood samples were obtained in only 50% of the running rats. Animals were then killed by decapitation, and their brains were rapidly removed and placed on a chilled glass platform for dissection. The hypothalamus was identified and dissected according to the methods of Palkovits and Brownstein (27) and Glowinski and Iversen (14), snap-frozen in liquid nitrogen, and stored at −80° for subsequent catechol analysis. Abdominal adipose tissue (from both the intra- and retroperitoneal depots) was removed and weighed.

On the day of catechol analysis, brain samples were thawed and accurately weighed before being homogenized on ice in 0.5 ml of 0.4 M perchloric acid containing 0.01% EDTA and a known amount of the internal standard 3,4-dihydroxybenzylamine. The homogenate was then rapidly centrifuged, and the supernatant was collected for subsequent neurochemical analysis. Catechols were extracted from the perchloric acid supernatant, and also from plasma, with alumina adsorption, separated by high-performance liquid chromatography, and the amounts were quantified by electrochemical detection according to previously described methods (22). The chromatographic system consisted of a model 480 high-pressure pump, model Gina autosampler, model STH 585 column oven, Chromelone 3.03 chromography data system (Gynkotek, Germering, Germany), model 5100A coulometric detector equipped with a model 5021 conditioning cell and a model 5011 analytical cell (Environmental Sciences Associates), and a 25-cm Altex Ultrasphere column (ODS 4.6 mm x 25 cm, 5-μm particle size, Beckman Instruments). Analysis was performed at 24°C with the operating potentials set at +0.35 V for the guard cell and −0.35 and +0.29 V for detectors 1 and 2, respectively. All measurements were made by using the oxidizing potential applied at detector 2, and compounds in plasma and brain extracts were identified by their retention behavior compared with that of authentic standard solutions. The intra-assay coefficients of variation were ±2% for norepinephrine, ±2% for DOPA, and ±2% for DHPG. All samples were analyzed in a single-batch analysis.

All results, unless otherwise specified, are expressed as means ± SE. Comparisons among groups were evaluated by using one-way analysis of variance. Relationships among variables were evaluated with either least squares linear or polynomial regression analysis. The null hypothesis was rejected at P < 0.05.

RESULTS

No discernible difference occurred in the running activity between the two groups of exercising animals, other than that one group ran for a longer period of time (Fig. 1). Peak running distance was achieved after ~3 wk. This level of exercise was maintained throughout the duration of the experiment, with the average distance run per day being 7.0 ± 0.8 km in the 6-wk group and 7.8 ± 0.7 km in the 11-wk group. There was no significant difference between the groups in the distance run on the day preceding death (8.9 ± 1.1 km in the 6-wk runners and 6.8 ± 1.5 km in the 11-wk animals). When compared with the sedentary control animals, exercise resulted in a marked diminution in abdominal fat mass (Table 1), the magnitude of which was similar in the two groups of running animals. The distance run, rather than the duration of exercise, was the stronger predictor of body fat [fat mass = −0.4 (average distance per day) + 6.7; r = 0.83, P < 0.01]. Although there was a tendency for the plasma levels of norepinephrine and DHPG to be lower in trained animals, the plasma concentration of DOPA was similar in all animals examined (Table 1). The animals’ degree of adiposity was significantly related, in a linear fashion, to their plasma DHPG concentration (fat mass = 0.7x + 5.7; r = 0.61, P = 0.02). Plasma norepinephrine levels displayed a tendency to be linked with abdominal fat mass (r = 0.43, P = 0.1).

Consistent with a possible reduction in rates of synthesis, release, and intraneuronal metabolism of norepinephrine, the concentration of norepinephrine, DHPG, and DOPA in hypothalamic tissue was substantially reduced in trained animals, with the incremental reduction in DHPG being greatest (Fig. 2). Although the duration of exercise bore no influence on the tissue content of these compounds, a trend existed for a negative relationship between the average distance
run per day and the hypothalamic concentration of norepinephrine \((y = -83x + 2,309; r = 0.34)\). In all animals examined, a strong association existed between the hypothalamic norepinephrine content and the plasma concentration of both norepinephrine \((y = 0.003x + 2.6; r = 0.58, P < 0.05)\) and DHPG \((y = 0.003x + 4.5; r = 0.65, P = 0.01)\).

Examination of the hypothalamic data in terms of the animal's fat mass provided evidence linking body composition with norepinephrine in the hypothalamus. The relationship between fat mass and hypothalamic norepinephrine content was best described via the second-order polynomial expression \(y = 63x^2 - 1,180x + 137x^2\) (Fig 3; \(r = 0.83, P < 0.001\)). A similar relationship between the concentration of DHPG in the hypothalamus and abdominal fat mass was also ascertained \((y = 20x^2 - 140x + 20x^2; r = 0.79, P > 0.001)\).

**DISCUSSION**

The popularity of regular exercise as a therapeutic tool has grown because of its beneficial influence on cardiovascular health and perceived positive effect on affective behavior. In an effort to understand the mechanisms responsible for such responses, a variety of animal models has been established, many of which may be unsuitable for examining brain monoaminergic function, given that the stimulus designed to initiate running activity constitutes a stress that may independently influence central nervous system neuronal function. Hoffmann et al. (15) and Dunne and colleagues (6) recently circumvented this problem by examining brain neurotransmitter levels in voluntarily exercising rats. Taken together, these results suggest that voluntary exercise is associated with region- and neurotransmitter-specific alterations in brain monoaminergic function. In the study by Dunne et al., significant increases in norepinephrine content in the pons-medulla region and the spinal cord are documented, and, interestingly, the addition of a “treadmill”-running group in their experimental design permitted them to conclude that voluntary and forced running paradigms do not elicit identical alterations in brain noradrenergic function.

To this juncture, though, there appears to have been little effort directed toward the examination of hypothalamic function in the voluntary exercise rat model.

Given its extensive neuronal circuitry projecting to autonomic premotor nuclei such as the A5 noradrenergic cell group and the rostral ventrolateral medulla (13), the hypothalamus may play a pivotal role in the regulation of autonomic reflexes and flight-or-flight responses involving autonomic activation (17). Indeed, stimulation of the paraventricular nucleus of the hypothalamus results in sympathetic nervous activation (20), and a reciprocal relationship between blood pressure and norepinephrine overflow from the paraventricular nucleus exists (29). Woo and colleagues (35) have also demonstrated enhanced norepinephrine release from the paraventricular nucleus in spontaneously hypertensive rats and propose that deranged modulation of noradrenergic transmission by neuropeptide Y is implicit in the enhanced hypothalamic noradrenergic activity, blood pressure elevation, and sympathetic activation (30, 32) seen in these animals. Of course, to consider these observations relevant in the present situation, one must assume that our exercising animals exhibited a lower sympathetic tone than did their sedentary counterparts and that the diminished concentrations of norepinephrine, DOPA, and DHPG within the hypothalamus in exercising animals are a reflection of a reduction in noradrenergic neuronal activity.

Consistent with a possible modulation in sympathetic nervous function, previous reports have documented an enhancement of cardiovascular performance in spontaneously hypertensive rats exposed to...
5–6 wk of voluntary exercise training, a paradigm similar to that used in the present report (11, 16). The tendency for plasma levels of norepinephrine, DOPA, and DHPG to be lower in our running animals, coupled with previously published reports documenting a reduction in total body sympathetic activity after chronic exercise training in humans (23, 25), is strong grounds for supporting that sympathetic nervous activity was reduced in our exercising animals. Whether such a response is a consequence of the exercise training per se, a reflex response to cardiopulmonary baroreceptor afferent input with training (4), or an attenuation of stress-induced elevations in blood pressure (2) remains uncertain. Although tissue concentrations of norepinephrine alone are not necessarily instructive as to the status of neurotransmitter release rates, especially given that the effects of exercise training on tissue catecholamine levels are site specific, the combined measurements of the putative transmitter along with its precursor and principal intraneuronal metabolite lend more weight to such determinations. Clearly, though, more rigorous experimentation combining techniques such as in vivo voltametry, dialysis, or direct nerve recording with pharmacological interventions is required before we can unequivocally state that voluntary exercise is associated with a diminished noradrenergic neuronal activity within the hypothalamus.

Our observation of a highly significant association between the hypothalamic norepinephrine content and the absolute abdominal fat mass is not without precedent. Indeed, the afferent signals that evoke changes in energy intake with regard to body weight regulation are presumed to arise partly from body stores, with the most likely candidate being adipose tissue depots. Initially espoused in Kennedy’s lipoat or apidost theory (21), this concept has gained increased credibility with the recent sequencing of the mouse obese gene and its human homologue (37). Considering the heterogeneity of sympathetic nervous activation in the obese state (33) and in response to exercise training (23), where clinically relevant regional alterations in sympathetic nervous activity may occur in the absence of changes in global indexes of sympathetic function, it is difficult to reconcile our results into a unifying hypothesis. Clearly, however, the intriguing nature of our data warrants speculation as to their possible significance.

Our demonstration of a positive relationship between the plasma and hypothalamic norepinephrine levels provides presumptive evidence of an association between noradrenergic neuronal activity of the hypothalamus and sympathetic nervous function. The observation that abdominal fat mass is closely linked with norepinephrine in the hypothalamus raises the possibility that alterations in body fat stores provide an afferent signal linking hypothalamic function and the activity of the sympathetic nervous system. It has recently been demonstrated that the obese gene product leptin enters the brain via a saturable mechanism (1) and acts, purportedly via the paraventricular nucleus of the hypothalamus (36), to increase energy expenditure and reduce body fat stores, effects that could, at least in part, be explained via alterations in sympathetic nervous system-mediated thermogenesis. Given the pronounced hypothalamic innervation from ascending noradrenergic projections (28), it would appear that there is scope for leptin to exert its effects via either direct or indirect mediation of hypothalamic noradrenergic neurotransmission. Although our parallel observations of a reduced hypothalamic content of norepinephrine and a markedly diminished abdominal fat mass in exercise-trained animals provide some support for the hypothesis of a feedback loop among body fat stores, the hypothalamus, and sympathetic nervous control, the interpretation of our data is somewhat difficult given the polynomial character of our observed relationship. Given that the animals with the lowest abdominal fat mass were those that ran the farthest, it is of note that exercise training has been shown to downregulate the expression of the obese gene (12, 38); whether the extent of such downregulation is emphasized by the level of training remains to be seen. Another possible explanation for the disparity in hypothalamic norepinephrine among our exercising animals may rest in the myriad of functions subserved by the hypothalamus. Contrary to what is observed in the paraventricular nucleus, noradrenergic processes in many other regions of the hypothalamus tend to exert the opposite effects (3); hence, the higher hypothalamic norepinephrine content seen in those exercising animals with the lowest levels of abdominal fat may reflect a predominance of noradrenergic activity in hypothalamic regions removed from the paraventricular nucleus. Clearly, though, further experimentation utilizing more precise methodology is necessary to unequivocally unravel the intricacies governing exercise, body composition, hypothalamic function, and the sympathetic nervous system.

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