Hemodynamic effects of the use of oral snuff

The hemodynamic effects during rest and exercise of oral snuff were investigated in an open, placebo-controlled study of nine habitual users of oral snuff. Blood pressure, heart rate, and central hemodynamics were measured noninvasively. Plasma concentrations of nicotine, cotinine, norepinephrine, and epinephrine, as well as neuropeptide Y–like immunoreactivity were measured before and after snuff intake during rest and exercise. Snuff intake induced a significant increase in heart rate and blood pressure and a decrease in stroke volume during rest. Hemodynamic changes were unrelated to nicotine or cotinine concentrations. Resting levels of norepinephrine and neuropeptide Y–like immunoreactivity were similar with or without snuff, whereas epinephrine was slightly increased 30 minutes after snuff intake. The exercise-induced increase in norepinephrine and neuropeptide Y–like immunoreactivity did not differ between the days subjects received snuff and the days they received placebo. In contrast, maximum work load was associated with a slight increase in circulating epinephrine after snuff intake. The findings suggest that snuff intake is associated with significant hemodynamic effects during rest but not during exercise. These effects could not be readily explained by activation of the sympathetic nervous system. (CLIN PHARMACOL THER 1992;52:394-401.)

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The use of oral snuff has increased significantly in a global perspective over the last two decades[1,2] and there has also been a shift in the profile of consumers. Users of oral snuff were previously found more commonly among older men, whereas today's snuff users are mostly young men.[3,4] National Swedish statistics covering the time period 1985 to 1987 report among males from 16 to 24 years of age, 31% are habitual users of oral snuff.[5]

The obvious hazardous health consequences of snuff dipping involve local carcinogenic effects of the oral mucosa.[6] However, possible additional short- and long-term cardiovascular side effects have recently been recognized.[7-9] Cigarette smoking is associated with short-term changes in heart rate and blood pressure[8] but not with sustained hypertension.[10] Previous studies dealing with the short-term hemodynamic effects of snuff dipping have shown significant effects on blood pressure and heart rate during rest,[8,9] whereas the cardiovascular effects during dynamic exercise remain to be clarified. In addition, there have been reports that claim an association between snuff dipping and hypertension.[7] The mechanism behind this association is still not established.

Snuff contains high amounts of nicotine that is readily absorbed across the oral mucosa.[11] The total average nicotine exposure after oral snuff intake may be similar to or even higher than that seen after cigarette smoking.[8] It may be assumed that the major hemodynamic effects of tobacco use may be directly attributable to the autonomic nervous effects induced by nicotine. Cigarette smoking has been associated with increased circulating levels of norepinephrine and epinephrine.[12,13] However, the effects of unburned tobacco on circulating catecholamine levels has, to our knowledge, not been investigated previously.

This study was undertaken to further investigate the short-term hemodynamic effects of snuff dipping during rest and dynamic exercise in healthy habitual users of oral snuff. Moreover, we wanted to investigate the possible relation between snuff-induced hemodynamic effects and the circulating levels of catecholamines, as well as the peripheral noradrenergic cotransmittor neuropeptide Y.

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MATERIAL AND METHODS

Nine healthy volunteers (eight males and one female) with a mean age of 27 years (age range, 25 to 31 years) were chosen for the study. All subjects had previous experience with oral snuff, and all but one were habitual users of oral snuff. All experiments were carried out on 2 different experimental days separated in time by 2 to 3 weeks. Subjects were randomly allocated to the order of the experimental days. One experimental day involved snuff intake, whereas the alternative day served as a control day. Except for snuff intake, all experimental procedures were identical on the 2 experimental days. The subjects were asked to refrain from snuff use for a minimum of 9 hours before the start of the experiment. The study protocol was approved of by the Ethics Committee of the Medical Faculty, University of Göteborg, Göteborg, Sweden.

All subjects arrived in the laboratory at 9 AM after a light breakfast without tea or coffee. All studies were performed with the subjects in the supine position except for the isometric and dynamic work and the cold pressor test, which were performed while the subjects were sitting. Heart rate was monitored by standard limb ECG leads, and systolic and diastolic blood pressures (Korotkoff phases I and V) were measured by a single observer with a standard mercury sphygmomanometer placed on the right arm of each subject. Stroke volume was determined noninvasively with impedance cardiography. A comprehensive description of the impedance cardiograph and the techniques used for determination of stroke volume are described elsewhere.[14] Cardiac output was calculated by multiplying stroke volume by the heart rate obtained from an ECG.

On the experimental day that involved tobacco, snuff (2.5 gm, commercial Swedish brand) was placed in the buccal sulcus under the upper lip after an initial resting period of 10 minutes (−10 to 0 minutes). The administered amount of snuff contained approximately 12.5 mg nicotine according to previously performed assays (data not presented). All recordings were performed at 0, 15, and 30 minutes after snuff intake. At 30 minutes, subjects were placed in the sitting position and new baseline values were obtained 5 minutes later. This was followed by an isometric exercise test during which blood pressure, heart rate, and an impedance cardiogram were recorded after a dominant hand handgrip at 30% of maximum voluntary contraction for 3 minutes. After an additional 30 minutes of supine rest, a similar sequential procedure was followed with the subjects in the sitting position for the cold pressor test. The left arm of each subject was lowered into and kept in a container with ice water for 3 minutes. All recording procedures were repeated at the end of the test. After an additional 30 minutes in the supine resting position, a final recording of all parameters was obtained. The oral cavity was rinsed and a light meal was served. Blood samples were obtained from an indwelling cannula placed in a superficial brachial vein of the right arm of each subject. Blood for subsequent analysis of plasma hormones, nicotine, and cotinine were withdrawn simultaneously with all recordings of hemodynamic variables.

Table I. Average plasma concentrations (±SEM) of nicotine and cotinine after snuff intake during supine rest*

Time (min)	*Nicotine (ng/ml)*	*Cotinine (ng/ml)*
0	0.3 ± 0.2	117.1 ± 37.1
15	7.7 ± 1.1	106.0 ± 32.0
30	11.2 ± 1.2	109.8 ± 32.7
70	16.1 ± 1.7	109.4 ± 29.5
110	20.9 ± 2.0	120.6 ± 30.7
140	16.5 ± 1.9	126.3 ± 28.2

*Values from nine subjects are shown.

A second dose of snuff (2.5 gm) was given 1 hour after the meal. Baseline recordings and blood sampling were repeated in the supine position and the subjects were moved to the sitting position for the dynamic exercise test on an electrically braked bicycle ergonometer. The work load was increased in steps of 50 W at 2-minute intervals until it reached 200 W. Subjects were thereafter exercised to limit symptoms at maximum work load above 200 W. Two subjects on each test day did not reach the 200 W work load during the exercise tests. In these cases, recorded values from work loads up to 150 W were used for further calculations. Blood pressure recordings, heart rate recordings, and blood samples were obtained during supine and sitting rest and at each load. A final recording and blood sampling was made at 15 minutes of supine rest after exercise was terminated.

Blood samples were collected into EDTA tubes on ice water (±0° C) for the subsequent analysis of nicotine and cotinine, plasma catecholamines (norepinephrine and epinephrine) and neuropeptide Y. Blood for nicotine and cotinine analysis was sampled into sterile tubes. After cold centrifugation (+4° C) the separated plasma was stored at −70° C. Nicotine and cotinine were analyzed by gas chromatography with a capillary column and a nitrogen selective detector (limit of

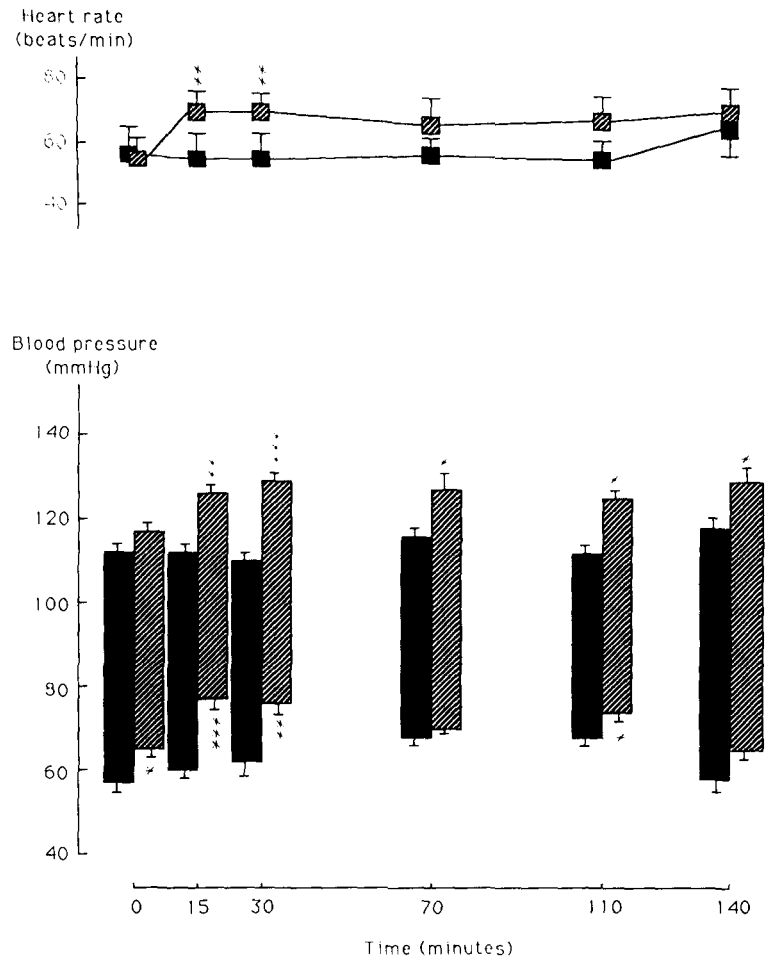

Fig. 1. Heart rate **(upper panel),** and systolic and diastolic blood pressures **(lower panel),** during control conditions and after snuff intake. Shown are mean values ± SEM in nine healthy volunteers after snuff intake *(shaded symbols)* or on control day *(solid symbols). Time scale* relates to time after snuff intake.*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

quantification for nicotine, 0.6 ng; limit of detection, 0.2 ng).

Plasma norepinephrine and epinephrine were analyzed in plasma by use of HPLC with electrochemical detection as described previously.[15]

Plasma neuropeptide Y–like immunoactivity was analyzed by radioimmunoassay according to Theodorsson-Norheim et al.[16] The antiserum used was raised in rabbits and showed no (<0.1%) cross-reactivity to structurally related peptides such as peptide YY or pancreatic polypeptide.

The Wilcoxon rank sum test was used for statistical comparison. The paired *t* test was used for comparison of cardiac output and stroke volume before and after snuff intake. A *p* value of 0.05 or less was considered significant. All findings are presented as mean values ± SEM.

RESULTS

Plasma concentrations of nicotine and cotinine. Seven of nine study subjects had measurable plasma cotinine levels (90.3 ± 27.7 ng/ml) at the start of experiment on the control day. The corresponding value on the study day was eight of nine subjects (117.1 ± 30.3 ng/ml). Nicotine was detected in two subjects at the control day and in three subjects on the study day. Pretreatment blood nicotine concentration averaged 0.25 ± 0.2 ng/ml and 0.34 ± 0.2 ng/ml on the 2 respective days. Values were consistent with overnight abstinence from snuff dipping.

Table II. Cardiac output and stroke volume during 110 minutes of supine rest on the control day or after snuff intake*

Time (min)	Stroke volume (ml)			Cardiac output (L/min)		
	Control	Snuff	p Value	Control	Snuff	p Value
0	128 ± 12	126 ± 11	—	7.01 ± 0.81	6.83 ± 0.58	—
15	128 ± 12	119 ± 10	NS	6.95 ± 0.96	8.17 ± 0.86	NS
30	133 ± 12	115 ± 8	<0.05	7.27 ± 0.88	7.89 ± 0.68	NS
70	128 ± 11	124 ± 11	NS	6.60 ± 0.75	8.06 ± 0.88	NS
110	130 ± 13	115 ± 8	<0.04	6.85 ± 0.75	7.20 ± 0.59	NS

NS, Not significant.
*Mean values ± SEM from nine subjects are shown; statistics by paired *t* test compared with control value at 0 minutes.

After snuff intake, the concentrations increased slowly to reach a plateau level of approximately 16 to 20 ng/ml at 70 to 110 minutes (Table I). After the second snuff intake, plasma nicotine levels continued to increase and reached 29.8 ± 3.8 ng/ml 60 minutes after intake (data not shown). Plasma levels of cotinine increased and reached 126.3 ± 28.2 ng/ml at 140 minutes after the first intake and 160.6 ± 29.6 ng/ml at 60 minutes after the second intake (Table I).

Plasma concentrations of nicotine or cotinine did not correlate to either hemodynamic data during rest or exercise, and they did not correlate to plasma catecholamine concentrations.

Hemodynamic effects during rest. Baseline diastolic blood pressure was higher ($p < 0.05$) on the study day compared with the control day. However, both systolic and diastolic blood pressure were markedly increased after snuff intake (Fig. 1). The increase in systolic blood pressure seemed to remain throughout the 140-minute supine registration period after snuff exposure, whereas the increase in diastolic blood pressure had a shorter duration. Heart rate had increased approximately 25% 15 to 30 minutes after snuff administration (Fig. 1, *upper panel*). This increase remained during the study interval. Thus, peak hemodynamic effects were seen after 15 minutes when plasma nicotine levels had reached only approximately 30% to 50% of peak values. Cardiac output tended to increase after snuff intake (Table II), although this change did not reach the level of significance. Stroke volume did not change during the control day, whereas there was a significant decrease at 30 minutes ($p < 0.05$) and 110 minutes ($p < 0.04$) after snuff intake (Table II). However, these changes were small, and no defenite conclusions could be drawn from these data.

Hemodynamic effects during exercise. The dynamic exercise test was performed after a repeated snuff intake. There was no significant difference in the maximum work load reached on the control day and after snuff intake (210.0 ± 9.1 and 205.6 ± 8.0 W, respectively). Both heart rate and diastolic blood pressure, but not systolic blood pressure, increased significantly at the low work loads (heart rate, 50 and 100 W, $p < 0.01$ and < 0.05, respectively; diastolic blood pressure, 50 W, $p < 0.05$, Fig. 2). Fifteen minutes after termination of exercise, heart rate was higher after snuff intake ($p < 0.001$), whereas the blood pressure response was similar with and without snuff.

Blood pressure, but not heart rate, was significantly higher after snuff at the start of isometric exercise ($p < 0.05$, data not shown). The heart rate response to isometric exercise was slightly more pronounced after snuff, whereas the differences in blood pressure tended to disappear. Similarly, in the cold pressor test the increased heart rate after snuff remained. However, both the pressor and cardioacceleratory effects of the cold pressor test were small (data not shown).

Plasma concentrations of norepinephrine and epinephrine. Resting (Table III) or exercise norepinephrine (Fig. 3) values did not differ significantly between the experimental days. Plasma epinephrine was slightly higher 30 minutes after snuff intake compared with control, although no other differences appeared in plasma epinephrine during rest (Table III). Similarly, there was a significantly higher plasma epinephrine level at 30 minutes at 200 W work load (Fig. 3).

Plasma concentrations of neuropeptide Y-like immunoreactivity. There were similar concentrations in neuropeptide Y-like immunoreactivity in the subgroup of four subjects on the snuff intake and control days (Table III). Neuropeptide Y-like immunoreactivity increased at maximum work load and remained increased in both groups 15 minutes after termination of exercise (Fig. 3). Neuropeptide Y-like immunoreactivity levels were unrelated to any of the investigated hemodynamic changes.

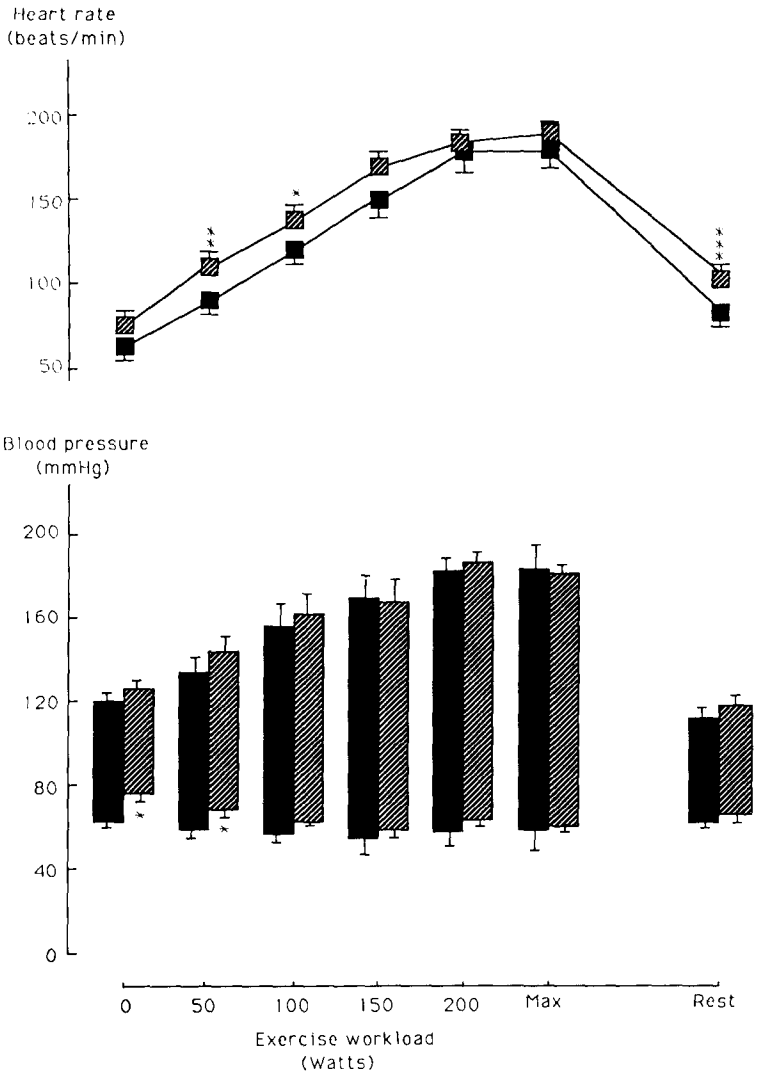

Fig. 2. Heart rate **(upper panel),** and systolic and diastolic blood pressures **(lower panel)** during graded dynamic exercise and subsequent supine rest. Shown are mean values ± SEM in nine healthy volunteers after snuff intake *(shaded bars)* or on control day *(solid bars). Time scale* relates to time after start of study. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

DISCUSSION

We have shown that the use of oral snuff may influence hemodynamics during rest and exercise in healthy normal volunteers. There were no significant elevations in the circulating levels of plasma catecholamines or neuropeptide Y–like immunoreactivity during rest. Plasma nicotine concentration did not correlate significantly to resting or exercise hemodynamics.

Nicotine is a weak base that is easily absorbed over the oral mucosa in users of oral snuff because of the alkaline pH of the tobacco product.[17] The time to reach maximum plasma concentration (t_{max}) is considerably shorter after cigarette smoking compared with the t_{max} after snuff dipping, whereas the average peak plasma concentration after oral snuff dipping is equal to or lower than that observed after cigarette smoking. Considering the normal exposure time for cigarettes or oral snuff, this will result in a far higher total nicotine exposure after snuff intake compared with that after smoking. From a consumer point of view, low consumption of smokeless tobacco (<10 gm/day) results in a total daily nicotine exposure, corresponding to roughly 130 to 250 mg nicotine, whereas a cigarette

Table III. Resting plasma concentrations of norepinephrine, epinephrine, and neuropeptide Y–like immunoreactivity during rest and exercise, with and without snuff*

	Norepinephrine (ng/ml)			*Epinephrine (ng/ml)*			*Neuropeptide Y–like immunoreactivity (pmol/ml)*		
Rest (min after snuff)	*Snuff (n = 9)*	*Control (n = 9)*	*p Value*	*Snuff (n = 9)*	*Control (n = 9)*	*p Value*	*Snuff (n = 4)*	*Control (n = 4)*	*p Value*
0	0.19 ± 0.04	0.15 ± 0.03	NS	0.04 ± 0.01	0.07 ± 0.04	NS	37.3 ± 4.8	26.7 ± 1.1	NS
15	0.15 ± 0.03	0.17 ± 0.03	NS	0.05 ± 0.01	0.05 ± 0.01	NS	41.7 ± 3.0	25.9 ± 4.2	NS
30	0.16 ± 0.03	0.17 ± 0.03	NS	0.07 ± 0.02	0.04 ± 0.01	0.05	32.1 ± 6.0	24.8 ± 1.6	NS
70	0.17 ± 0.05	0.17 ± 0.03	NS	0.06 ± 0.02	0.05 ± 0.02	NS	38.8 ± 4.5	28.9 ± 3.7	NS
110	0.23 ± 0.06	0.18 ± 0.04	NS	0.06 ± 0.02	0.03 ± 0.01	NS	37.8 ± 6.3	34.0 ± 1.5	NS
140	0.26 ± 0.07	0.22 ± 0.03	NS	0.06 ± 0.03	0.04 ± 0.01	NS	26.2 ± 2.3	30.1 ± 2.3	NS

*Shown are mean values ± SEM from nine subjects (norepinephrine and epinephrine) and four subjects (neuropeptide Y–like immunoreactivity); statistics by the Wilcoxon rank sum test.

smoker who smokes 20 cigarettes daily is exposed to 180 mg nicotine.[18]

The average plasma nicotine concentration after 24 hours abstinence in this study (20.9 ng/ml) was in accordance with data presented earlier in users of moist oral snuff.[17] Moreover, the plasma concentrations were stable over the two study periods in spite of the relatively short half-life of nicotine (2 hours[11]). This appears to be a result of the constant exposure during the experiment whereby rate of elimination is balanced by rate of absorbtion. Plasma levels of cotinine, the major metabolite of nicotine, were high at start of the study, reflecting its long plasma half-life.[19] The levels, which were in accordance with earlier reported data,[20] remained stable at steady state throughout the study. Cotinine has earlier been reported to be devoid of biologic activity,[21] and it does not appear to be likely that the findings obtained during both the experimental and control day were influenced by the high cotinine levels.

Smokeless tobacco has been associated with increased diastolic blood pressure in epidemiologic studies that involved healthy habitual users.[7,22] Heart rate and blood pressure increased significantly as a consequence of nicotine uptake when either a nicotine toothpick or nicotine gum was chewed.[23] The acute hemodynamic effects of nicotine seem to differ from previously reported long-term effects. All tobacco use, including smoking or intake of smokeless tobacco, was associated with a rapid increase in heart rate, systolic blood pressure, and diastolic blood pressure in habitual users.[8]

The present findings support earlier hemodynamic data from subjects during rest after smokeless tobacco intake.[24] The pressor and cardioacceleratory response were rapid in onset and remained for almost two hours. Thus it is interesting to note that hemodynamic effects already peaked during the rising phase of the nicotine plasma concentration curve. The almost immediate hemodynamic response is also in accordance with other reports.[25] Although cardiac output tended to increase, this change was insignificant, possibly because that cardioacceleration was compensated for by a decrease in stroke volume after snuff exposure. In view of the pressor response, this indicates that total peripheral resistance was increased. Clearly, this data has to be interpreted with some caution because all subjects in this study were habitual users of oral snuff. Therefore they may potentially have already developed functional or structural cardiovascular changes, resulting in a modified short-term hemodynamic response to single-dose snuff intake. The significant hemodynamic effects during rest were rapidly blunted after dynamic exercise, and maximum exercise tolerability was similar with or without snuff.

The exact mechanism(s) by which smokeless tobacco causes both short-term and sustained increases in blood pressure and heart rate remain unresolved. One possible explanation may relate to the more prolonged nicotine exposure that is associated with the use of oral tobacco. Other possible explanations involve the high sodium content of smokeless tobacco,[26] which may act to elevate blood pressure after ingestion and absorbtion in persons who swallow the juices during use. Indeed, a higher urinary sodium excretion has been shown in users of oral snuff compared with cigarette smokers.[26] Finally, it has also been claimed that nicotine intake results in an activation of the sympathetic nervous system after all types of tobacco consumption,[25] presumably by way of a direct nicotine-induced activation of the sympathetic ganglia. Such an interpretation may be supported by the slight changes in plasma epinephrine detected in this study. The hemodynamic effects may primarily be

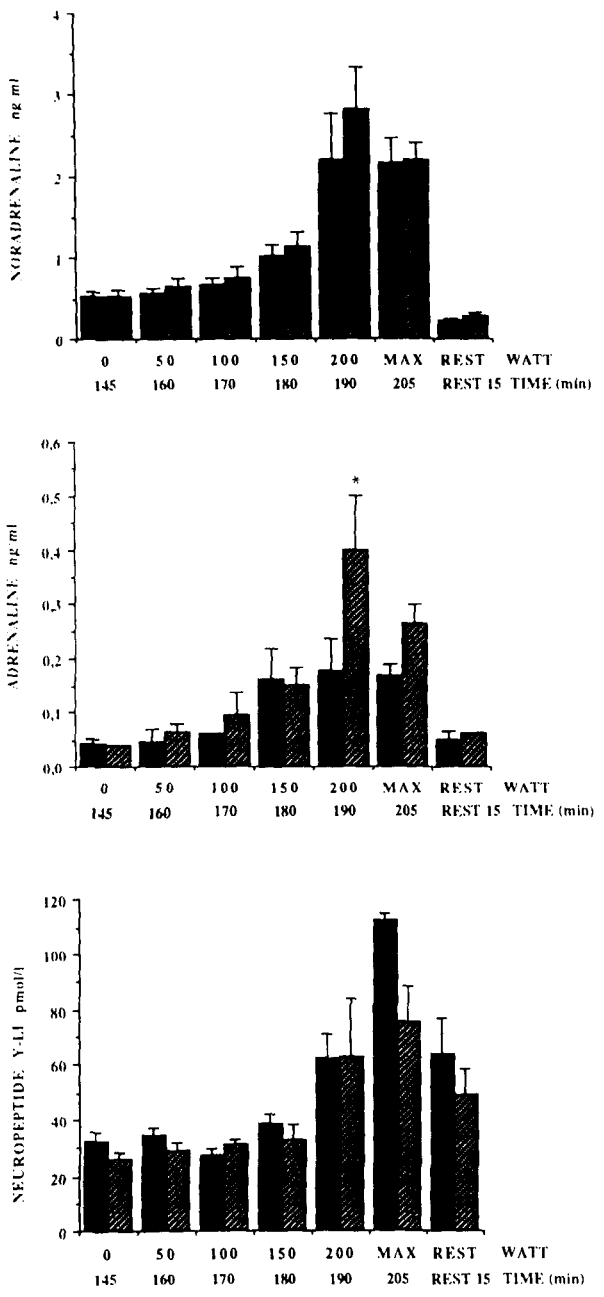

Fig. 3. Plasma norepinephrine (noradrenaline, **upper panel**) epinephrine (adrenaline, **middle panel**), and neuropeptide Y–like immunoreactivity (neuropeptide Y–LI, **lower panel**) during graded dynamic exercise and subsequent supine rest. Shown are mean values ± SEM in nine healthy volunteers after snuff intake *(shaded bars)* or on control day *(filled bars)*. *Time scale* relates to time after start of study. $*p < 0.05$.

an effect of norepinephrine release from adrenergic axon terminals, inasmuch as they preceded measurable levels of circulating catecholamines in cigarette smokers.[25] A sympathetic activation may also explain several of the long-term hemodynamic risk factors associated with smoking,[27] such as peripheral vascular effects, increased thrombocyte aggregation, and effects on fat and carbohydrate metabolism.

To our knowledge, there are no previous reports relating increased levels of circulating catecholamines to snuff intake. In this study, plasma norepinephrine and epinephrine were similar during the experimental and control days. Still, the hemodynamic effects after snuff intake were similar to those described elsewhere after snuff intake. Thus the present findings would lead us to question whether the hemodynamic effects were attributable to increased sympathoadrenergic activation. One additional way to study adrenergic activation relates to neuropeptide Y. Neuropeptide Y is a peptide with 36 amino acid residues that has recently been shown to be colocalized and coreleased with norepinephrine from peripheral adrenergic nerves.[28] Neuropeptide Y may be released together with norepinephrine after electrical nerve stimulation[29] or dynamic exercise.[30] Neuropeptide Y may also enhance the vascular α-receptor–stimulating effect of norepinephrine, and its biologic significance may relate to the long half-life of its biologic response after release. However, circulating neuropeptide Y–like immunoreactivity remained unaffected after snuff intake.

We conclude that oral snuff intake resulted in short-term hemodynamic effects unrelated to circulating catecholamine or neuropeptide Y–like immunoreactivity levels. These effects were blunted during exercise and may be the result of direct neurogenic sympathoadrenergic activation or other unidentified modulatory mechanisms in cardiovascular hemodynamic control.

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