Peripheral Afferent Stimulation of Decentralized Sympathetic Neurons Activates Lipolysis in Spinal Cord–Injured Subjects

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Spinal cord–injured (SCI) subjects exhibit a normal lipolytic rate despite the failure of centrally mediated sympathoexcitatory stimuli to activate lipolysis. Peripheral afferent stimulation below the lesion level induces an exaggerated autonomic reaction in SCI with lesion levels above T5, ie, so-called autonomic dysreflexia. The metabolic effects of induced dysreflexia were investigated in five SCI subjects (age, 35 ± 8 years; duration of paresis, 15 ± 7.5 years [mean ± SD]; lesion level, T3 to T4, n = 2, C7, n = 3) following bladder stimulation. Subcutaneous glycerol concentrations were measured by microdialysis above and below the lesion level. Diurnal plasma noradrenaline (NA) and adrenaline levels were continuously monitored in seven SCI subjects (lesion level T3 to T4, n = 2; C4 to C7, n = 5). Bladder stimulation resulted in an increased mean arterial pressure ([MAP] 81 ± 8 to 114 ± 11 mm Hg, $P < .05$), a decreased heart rate (70 ± 3 to 54 ± 4 beats/min, $P < .05$), and an increased plasma NA (0.70 ± 0.49 *v* 3.27 ± 1.56 nmol/L, $P < .05$). Interstitial glycerol was increased in the decentralized region (89 ± 12 to 135 ± 21 μmol/L, $P < .05$), whereas no reaction was found in the centrally innervated region. Plasma concentrations of glycerol and insulin increased. Diurnal monitoring showed periods of increased plasma NA sufficient to induce lipolysis (>1.4 nmol/L) during 20% of the registration period. The data suggest that peripheral afferent stimulation below the lesion level increases NA release and activates lipolysis and that frequent episodes of activation are found in SCI subjects with tetraplegia or high paraplegia.

A NORMAL LIPOLYTIC RATE in subcutaneous adipose tissue has previously been demonstrated postabsorptively in spinal cord–injured (SCI) subjects with lesion levels of T1 to T5.[1] We were not able to demonstrate any differences between centrally innervated and decentralized adipose tissue evaluated by a microdialysis technique. This was intriguing since it is known that the sympathoadrenergic system is involved in the regulation of lipolysis,[2,3] and in the SCI subjects studied, the greater part of the sympathetic nervous system and the adrenal medulla were decentralized. When challenging the subjects with standardized centrally mediated sympathoexcitatory stimuli, no activation of lipolysis below the lesion level was found.[4]

At baseline conditions and during centrally mediated stimulation, hemodynamics, noradrenaline (NA) release,[5] and direct sympathetic nerve fiber recordings[6,7] demonstrate low levels of sympathetic activity in SCI. On the other hand, SCI subjects with lesion levels above T5 may exhibit so-called autonomic dysreflexia, manifested as a blood pressure increase, baroreceptor-mediated heart rate decrease, and plasma NA increase, indicating that peripheral afferent stimulation below the lesion level may activate the sympathetic nervous system to a substantial degree.[8-11] The present study addresses the question of whether such peripheral stimulation also activates lipolysis. To test this notion, we performed a microdialysis study in SCI subjects in whom subcutaneous adipose tissue release of glycerol was determined at baseline and during provoked dysreflexia.

To evaluate the importance of peripheral afferent stimulation in maintaining a fairly normal lipolytic rate, the occurrence of peripheral stimulation has to be estimated. Since centrally mediated activation is known not to affect plasma NA levels in high paraplegics and tetraplegics, an increase in plasma NA may serve as an indicator of peripheral afferent stimulation of the decentralized sympathetic nervous system. Therefore, we performed 24-hour continuous monitoring of plasma NA and adrenaline in SCI subjects with a lesion level above T5. The following questions were addressed: (1) Do the widespread effects elicited during peripherally induced autonomic dysreflexia include stimulation of lipolysis?; and (2) Are there episodes of increased plasma NA during daily living in SCI subjects indicating significant peripheral afferent stimulation?

SUBJECTS AND METHODS

Subjects

Peripheral afferent stimulation in the form of bladder percussion over the lower abdominal wall was used to induce a dysreflexia reaction in a group of eight SCI subjects. The stimulation was successful in five subjects, who responded with a dysreflexia reaction characterized by an increase in mean arterial pressure (MAP), a decrease in heart rate, and an increase in plasma NA, in line with the findings of other investigators.[10] Three nonresponding SCI subjects were excluded from the analysis and are not reported. Subject characteristics are listed in Table 1. Four subjects had their primary care in the Göteborg region, and one was recruited from Stockholm. Since bladder stimulation does not induce any reaction among neurologically intact individuals,[12] no control group was investigated.

All five responders were healthy, except one who had a minor pressure sore on his left foot. One member of the group was medicating with anticholinergic drugs to stem urinary leakage. All were instructed to refrain from tobacco use 12 hours before investigation. Bladder emptying was managed by clean intermittent self-catheterization in all subjects. The segmental level of loss of sympathetic neural function was established by a forced perspiration test,[1,13] and was found to correspond closely to the loss of somatosensory function (±1 level). Body composition was determined by dual-energy X-ray absorptiometric

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Table 1. Peripheral Afferent Stimulation: Subject Characteristics

Characteristic	Value
No. of subjects	5
Sex (female/male)	0/5
Age (yr)	35 ± 8
Weight (kg)	86.6 ± 14.3
Lean body mass (kg)	54.9 ± 5.4
Fat tissue mass (kg)	27.7 ± 10.7
Lesion level	
T3-T4	2
C7	3 (1 subject sensory incomplete)
Duration of paresis (yr)	15 (range, 6-26)

scanning using a Lunar DPX (L) scanner (Scanexport Medical, Helsingborg, Sweden).[14] Body composition was determined as lean body mass, fat tissue mass (Table 1), and bone mineral content (not shown).

Three subjects from the responder group were included with four additional SCI subjects in a seven-subject group for 24-hour continuous monitoring of plasma NA. All were healthy, except one patient who had an ingrown toe nail (no. 3 in Fig 1; for subject characteristics, see Table 2 and Fig 1). One of the subjects (no. 5 in Fig 1) smoked during the investigation period, and this may have affected his NA release, but it did not affect the total group values.

The Ethical Committee of Sahlgrenska University Hospital, Göteborg, Sweden, approved the study, and all subjects provided informed consent.

Study Protocols

Peripheral afferent stimulation. The subjects arrived at the laboratory in the morning after an overnight fast and were investigated in the supine position. Subjects were instructed not to empty their urinary bladder during a 4-hour period before investigation. A polyethylene catheter was placed into an antecubital vein for blood sampling. The arm was heated with electrical pads to permit sampling of arterialized venous blood.[15] Blood samples were immediately centrifuged, and the plasma was stored at −4°C. Plasma was analyzed for glucose, insulin, glycerol, free fatty acids (FFAs), lactate, NA, and adrenaline.

Microdialysis catheters (30 × 0.3 mm Cuprophan B4 AH, 3000 MV cutoff; Cobe, Denver, CO) were placed in the left abdominal subcutaneous adipose tissue 5 cm lateral to the umbilicus (umbilical region) and in the subcutaneous adipose tissue 5 cm above the mamilla (clavicular region) corresponding to a level 5 cm proximal to the sympathetic disruption. The subjects thereby served as their own controls. The nylon tubing inlet of the microdialysis catheter was connected to a microinjection pump (Carnegie Medicine, Stockholm, Sweden). Saline with 2.5 mmol/L glucose was used to perfuse the system at a rate of 2.5 μL/min. After 30 minutes of equilibration, a calibration procedure was performed.[16]

The heart rate and continuous noninvasive blood pressure were monitored with the volume clamp technique (Finapres; Ohmeda Monitoring Systems, Englewood, CO), and resting values are presented as the mean value during the last 5 minutes before bladder stimulation. During bladder stimulation, mean values were calculated per minute. MAP was calculated as diastolic blood pressure + ⅓ (systolic − diastolic).

Bladder stimulation was performed for 5 to 6 minutes with 30 to 50 taps/min over the lower abdomen, a clinically established way to empty the urinary bladder in SCI subjects. Blood samples were collected immediately before the stimulation and after completion of the stimulation.

24-hour NA monitoring. Subjects arrived at the ward in the morning, and a thin cannula was inserted into a peripheral vein in the lower arm. The cannula was connected via a thin nylon tube to a pump device (Kowarski-Cormed, Medina, NY) placed on the forearm. Continuous blood sampling for 24 hours was started with an automatic blood withdrawal system at a flow rate of 6 mL/h.[17] Blood samples were collected in vials changed every 30 minutes. Blood samples were immediately centrifuged and subsequently stored at −80°C until analyzed. The subjects spent 24 hours in the ward without any food or activity restrictions, and sleeping was not disturbed by the exchange of vials.

Table 2. Continuous 24-Hour NA Monitoring: Subject Characteristics

Characteristic	Value
No. of subjects	7
Sex (female/male)	0/7
Age (yr)	31 ± 8
Lesion level	
T3-T4	2
C4-C7	5 (2 subjects sensory incomplete)
Duration of paresis (yr)	8 (range, 1-23)

Chemical Analyses

Glycerol was determined according to the method used by Laurell and Tibbling[18] and lactate was determined according to the method used by Loomis,[19] and aliquots were read on a spectrofluorometer. Glucose and FFAs were estimated enzymatically (Wako Chemicals, Neuss, Germany). The insulin level was measured with a radioimmunoassay technique, and NA and adrenaline were analyzed by a high-performance liquid chromatographic method with electrochemical detection.

Statistics

Values are presented as the mean ± SEM, except for subject characteristics, which are the mean ± SD. The Wilcoxon signed rank-sum test was used for comparisons between baseline and stimulation. P less than .05 was considered statistically significant.

RESULTS

Peripheral Afferent Stimulation

Bladder stimulation was followed by an increase in MAP from 81 ± 8 to 114 ± 11 mm Hg ($P < .05$), whereas heart rate decreased from 70 ± 3 to 54 ± 4 beats/min ($P < .05$). Plasma NA increased almost fivefold during stimulation (Table 3), whereas plasma adrenaline showed no increase (data not shown).

Interstitial glycerol below the lesion level in the umbilical region increased by 50% following bladder stimulation, whereas no reaction was found in the clavicular region. Activation of lipolysis was also apparent in plasma, where glycerol increased by one third, whereas FFAs showed a nonsignificant increase (Table 3). Insulin levels increased (Table 3), whereas plasma glucose and lactate levels were unaltered (data not shown).

Continuous Diurnal NA Sampling

Mean plasma NA in the 24-hour registration was 1.04 ± 0.03 nmol/L. Individual 24-hour profiles for NA are given in Fig 1. Twenty percent of the 30-minute samples showed NA levels more than 1.40 nmol/L (the level for which a control group showed activated lipolysis following mental stress[4]). Two subjects showed some adrenaline release (mean, 0.07; range, 0 to 0.26; and mean, 0.24; range, 0 to 0.34 nmol/L, respectively),

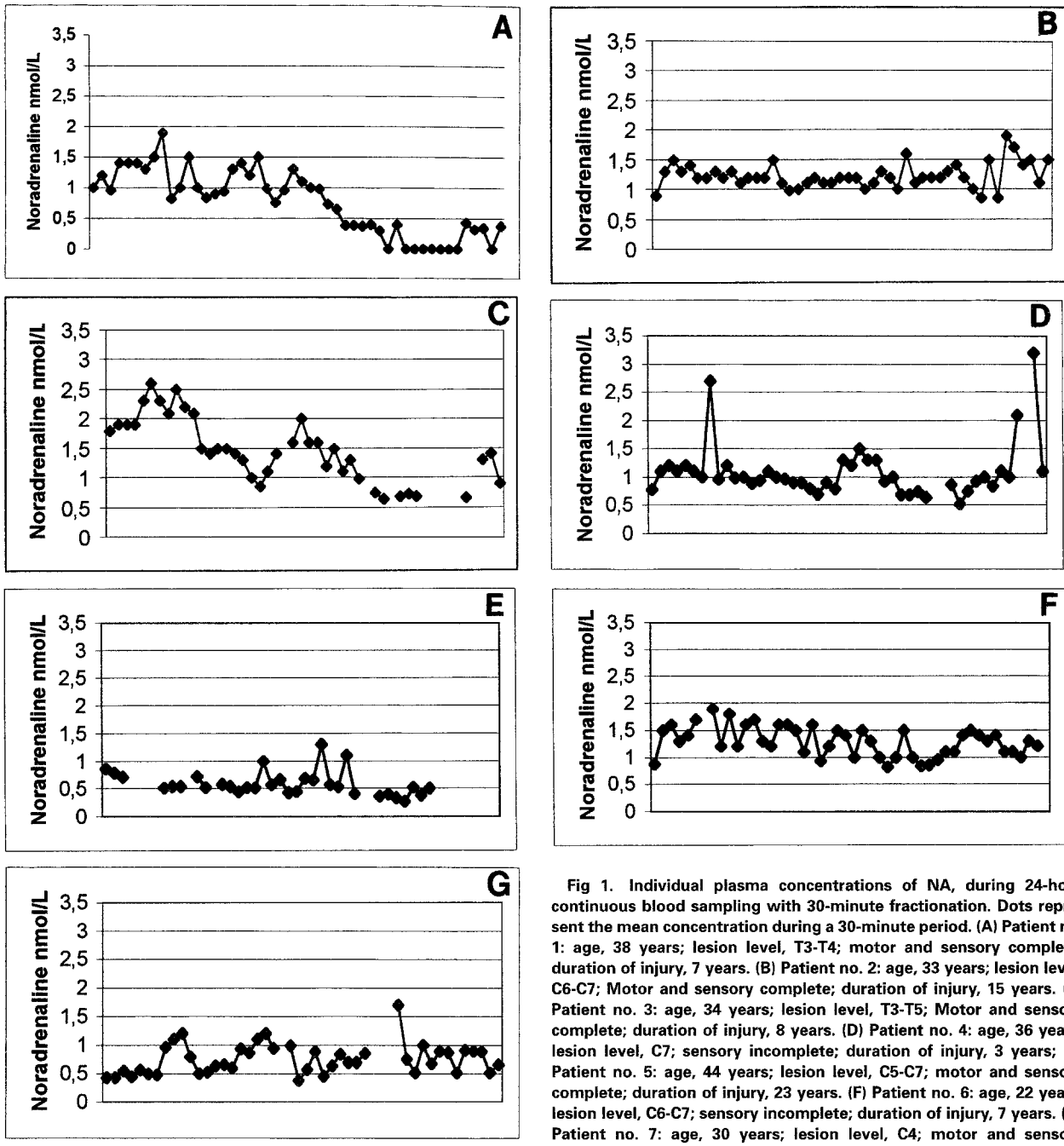

Fig 1. Individual plasma concentrations of NA, during 24-hour continuous blood sampling with 30-minute fractionation. Dots represent the mean concentration during a 30-minute period. (A) Patient no. 1: age, 38 years; lesion level, T3-T4; motor and sensory complete; duration of injury, 7 years. (B) Patient no. 2: age, 33 years; lesion level, C6-C7; Motor and sensory complete; duration of injury, 15 years. (C) Patient no. 3: age, 34 years; lesion level, T3-T5; Motor and sensory complete; duration of injury, 8 years. (D) Patient no. 4: age, 36 years; lesion level, C7; sensory incomplete; duration of injury, 3 years; (E) Patient no. 5: age, 44 years; lesion level, C5-C7; motor and sensory complete; duration of injury, 23 years. (F) Patient no. 6: age, 22 years; lesion level, C6-C7; sensory incomplete; duration of injury, 7 years. (G) Patient no. 7: age, 30 years; lesion level, C4; motor and sensory complete; duration of injury, 1 year.

whereas adrenaline was undetectable in the remaining five subjects.

DISCUSSION

Our main finding is that peripheral afferent stimulation of sympathetic nerves below the lesion level in SCI subjects, in addition to its well-known effects on blood pressure and heart rate, also activates lipolysis. The demonstration of frequent episodes of NA levels sufficient to activate lipolysis suggests that this peripheral mechanism may be of importance for the overall regulation of lipolysis in SCI.

Peripheral Afferent Stimulation

A significant increase in plasma NA in subjects with high paraplegia or tetraplegia is most likely attributable to peripheral afferent stimulation of sympathetic fibers below the lesion, since only a small part of the sympathetic nervous system is under central control in these subjects.[10] In support of this notion, we found no increase in NA release, or activation of lipolysis, during centrally mediated sympathoexcitatory stimuli in a previous investigation of this subject group.[4] In contrast, we found an increase in interstitial glycerol below the lesion level, as well as plasma glycerol, in all subjects for whom peripheral

Table 3. Peripheral Afferent Stimulation: Biochemical Profile (mean ± SEM)

Parameter	Baseline	Peripheral Afferent Stimulation
Interstitial glycerol (μmol/L)		
Umb	89 ± 12	135 ± 21*
Clav	113 ± 10	126 ± 42
Plasma glycerol (μmol/L)	91 ± 8	123 ± 16*
Plasma FFA (mmol/L)	0.8 ± 0.1	1.03 ± 0.1
Plasma insulin (pmol/L)	62 ± 9	75 ± 9*
Plasma NA (nmol/L)	0.70 ± 0.49	3.27 ± 1.56*

Abbreviations: Umb, umbilical, decentralized level; Clav, clavicular, sympathetically innervated level.
*$P < .05$.

afferent stimulation triggered a dysreflexia reaction in the present study. It may be noted that SCI subjects in general have a reduction in muscle mass and that adipose tissue mass is increased both absolutely[20] and relative to body weight.[4] Body composition in the present subject group was characterized without comparing the data against a matched control group (Table 1). However, comparison to previous data obtained in this laboratory suggests that the SCI subjects had an increased fat tissue mass.[4] Since the whole-body lipolytic rate is dependent on the interstitial fluid concentration of glycerol per kilogram fat mass, the data may lead to the conclusion that activation of lipolysis by peripheral stimulation contributes significantly to whole-body lipolysis, as indicated by the elevated plasma glycerol levels.

Activation of the decentralized sympathetic nervous system is, in all probability, caused by a spinal reflex arc. Morphological studies in animals have revealed alterations in sympathetic preganglionic neurons after experimental SCI.[21] Directly following a spinal transection, the dendrites of the decentralized sympathetic preganglionic nerves degenerated. Signs of afferent sprouting and new synapse formation in the same area followed. This posttraumatic neural remodeling may be of importance for the demonstrated pathological peripheral afferent sympathetic reflexes.

Bladder stimulation affected hemodynamics and lipolysis, whereas no adrenal reaction was detected. Consequently, the adrenal medulla does not appear to receive afferent input from visceral organs but relies exclusively on central control. This agrees well with the finding that hypoglycemia does not activate the adrenal medulla in SCI subjects.[22]

Continuous Diurnal NA Sampling

In contrast to studies in able-bodied subjects,[23,24] 24-hour plasma NA levels in our SCI group showed no diurnal variation, confirming the loss of blood pressure–related diurnal variations in SCI demonstrated by Krum et al.[25] The rapid normalization of NA levels within 5 to 10 minutes after bladder stimulation found in this study, as well as by others,[10] underlines the fact that continuous sampling yields more reliable information about NA release.

The mean diurnal NA value in SCI was, as expected,[10] lower than values found in the able-bodied.[23] However, it was higher than the basal values found in resting SCI[10] (and the present investigation). This finding may indicate that repeated peripheral afferent stimulation activates the sympathetic nervous system and contributes to the mean 24-hour level found. Nothing is known about the threshold for plasma NA activating lipolysis, but in previous studies plasma NA levels of 1.70 nmol/L[3] and 1.40 nmol/L[4] were found when lipolysis was activated by mental stress in able-bodied subjects. The SCI subjects presently investigated had plasma NA levels more than 1.40 nmol/L during 20% of the 30-minute sampling periods, indicating that lipolysis may have been stimulated for nearly 5 hours in a 24-hour period. The peripheral stimulation used by SCI subjects to increase performance during athletics ("boosting")[26] may partly rely on an increased supply of metabolic substrates by inducing lipolysis. This may serve to compensate for the previously demonstrated perturbed centrally mediated lipolytic mobilization following insulin-induced hypoglycemia,[27] as well as after centrally mediated sympathoexcitatory stimuli.[4]

In summary, SCI subjects exhibited an activation of lipolysis during peripheral afferent stimulation, in all probability mediated via activation of the sympathetic nervous system below the lesion level. Furthermore, the group demonstrated frequent periods of increased plasma NA during 24-hour continuous monitoring, reaching levels previously shown to activate lipolysis. These findings suggest that peripheral stimulation below the lesion level may compensate for the lack of central activation of lipolysis in SCI subjects, thereby maintaining an almost normal lipolytic rate in the group.

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