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Original Article

Different effect of locomotor exercise on the homogenate concentration of amino acids and monoamines in the rostral and caudal lumbar segments of the spinal cord in the rat

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Study design: The effect of long-term (4 weeks) moderate locomotor exercise on segmental distribution of glutamate (Glu), aspartate, gamma-aminobutyric acid, glycine (Gly), serotonin and noradrenaline in the spinal cord of adult rats was investigated.
Objectives: In light of the data showing modulation of some neurotransmitters in the low-lumbar segments of the rat due to physical exercise, our aim was to establish how segmentally specific is this effect with respect to neuroactive amino acids and monoamines.
Setting: Laboratory of Reinnervation Processes, Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland.
Methods: Amino acids and monoamines content was measured by means of HPLC in the whole tissue homogenate of the spinal cord in nonexercised and exercised rats.
Results: Glu and Gly homogenate concentration was the highest among all tested compounds. There was an intersegmental rostro-caudal gradient of concentration of neuroactive amino acids and monoamines, progressing caudally. Exercise modified this gradient exerting opposite effect on their concentration of amino acids and monoamines in the rostral and caudal lumbar segments.
Conclusion: Locomotor exercise leads to neurochemical remodeling of the spinal cord, which is differently manifested in the rostral and caudal lumbar segments of the spinal cord.
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Keywords: locomotor exercise; segmental gradient; neuroactive amino acids; monoamines

Introduction

Locomotor training in spinal animals improves the quality of walking, increasing the ability to support the weight of the body and recovery of plantar foot placement.[1–3] As beneficial effect of locomotor training has often been attributed to modulation of neurotransmitter systems in the spinal cord (for a review see Rossignol *et al*[4]), an understanding of neurochemical changes occurring in the spinal cord owing to locomotor exercise is of special importance.

Available data indicate that hindlimb locomotor activity modulates neurotransmitter concentration in the caudal lumbar segments.[5–7] Glutamate (Glu) level measured by means of microdialysis probe in the dorsal horn of L5 spinal segment increased in walking rat and returned to resting level soon after termination of locomotor activity.[6] Recently, it was proved that locomotor training leads to attenuation of gamma-aminobutyric acid (GABA) synthesis in the segments caudal to the lesion[7] suggesting a decrease in GABA-ergic transmission owing to the training.

The level of monoamines, measured in the ventral funiculus or in the ventral horn at L4 spinal segment of the rat by means of microdialysis probe, was modulated in a complex way during walking, depending on the location of the probe.[8,9] The concentration of 5-HT, 5-HIAA, dopamine and NA metabolite 3-methoxy-4-hydroxyphenylethyglycol (MHPG) increased in the

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Statement of ethics: Procedures involving animals and their care were conducted in conformity with the guidelines of the Ethical Council of the Nencki Institute, which are in compliance with the rules established by the Polish Council on Animal Care

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ventral funiculus as compared with pre- or post-exercise levels, whereas it decreased in the ventral horn compared to the resting levels, decreasing further shortly after exercise.[8,9]

Although the majority of data show transient changes in the caudal lumbar segments accompanying locomotor activity, it is possible that long-term locomotor training results in more persistent modulation of neurotransmitter system associated with remodeling of spinal network. To verify this hypothesis, we evaluated the effect of long-term, moderate locomotor training on the concentration of both amino acids and monoamines in the whole tissue homogenate of thoracic, lumbar and sacral spinal segments.

The data indicating that the network of interconnected spinal interneurons located in rostral lumbar segments of the rat may play prominent role in the control of spinally distributed locomotor network[10–14] prompted us to verify whether the neurochemical activity in rostral *versus* caudal lumbar segments is similarly modulated by long-term, moderate locomotor exercise.

Our results show that the homogenate concentration of amino acids and monoamines is lower in rostral than in caudal lumbar segments of the spinal cord of the adult rat. Moreover, neurochemical activity in these two parts of the lumbar segments is differently modulated owing to long-lasting locomotor exercise. These results are discussed in terms of functional diversity of the rostral and caudal lumbar segments in the control of locomotion.

Materials and methods

Animals
Twelve adult male Wistar rats, weighing 360–540 g at the end of the experiment, were used in the work described here. The animals were bred in the animal house of the Nencki Institute, Warsaw, Poland. They were given free access to water and pellet food and were housed under standard humidity and temperature, and 12 h light/dark cycle.

Behavioral training
Seven rats walked on a treadmill about 1000 m daily with the speed between 20 and 25 cm/s. The running distance corresponding to low daily running activity of adult rats during voluntary running in the running wheel has been chosen.[15] The locomotor training was carried out for 4 weeks, 5 days a week. Both the total daily walking distance and the speed of locomotion were gradually increased after the animals became accustomed to the treadmill. The daily training consisted of three to four 20-min walking sessions separated by about 1 h rest in the animal cages. The animals were rewarded with their preferred food after each session. The control group consisted of five animals that were never trained, but were handled and rewarded in the same way as the trained groups. After four experimental weeks, the trained animals gained their initial body weight by about 4%, whereas the non-trained by about 11%.

Tissue preparation
After 4 weeks, up to 2 h after the last running session, the rats were decapitated. Immediately after decapitation the corps were cooled, spinal cords were removed from the vertebral columns and divided into mid- and caudal thoracic (T), rostral lumbar (L1/2), caudal lumbar (L3–5) and sacral (S) segments. Isolated tissues were weighted and frozen (−70°C) until HPLC analysis. Immediately prior HPLC assay, tissue samples were sonicated in 0.1 M perchloric acid solution containing 0.4 mM sodium metabisulfite and centrifuged at $7000 \times g$. Supernatants were filtered (Spartan 3/0.2 PA nylon syringe filter, Schleicher &Schuell) and kept on ice till injection into HPLC system.

HPLC analysis
The levels of amino acids: Glu, aspartate (Asp), glycine (Gly), GABA, and monoamines: noradrenaline (NA), serotonin (5-HT), and 5-HT metabolite 5-HIAA were measured simultaneously in each sample, using two different HPLC systems (Merck-Hitachi). All measurements were carried out in quadruplicates.

Amino acids and monoamines separation
Before injection the samples used for amino-acid assay were automatically derivatized with ortho-phtaldialdehyde (Merck, Germany) and β-mercaptoethanol (Sigma) in 0.5 M borate buffer with methanol (1:9) and injected with the aid of autosampler (Merck-Hitachi, LaChrom, L-7250). The sample size was 20 μl. The separation of amino acids was carried out using Li Chromspher 18 RP $250 \times 4.6 \times 5$ column and a mobile phase, which was a binary eluent of 50 mM sodium acetate (pH 7.0) and methanol, under gradient conditions (CH_3OH from 26 to 40% during 0.5 h). Column temperature during separation was maintained at 35°C. The flow rate was 1.0 ml/min. For detection of separated amino acids fluorescence detector was used (Merck-Hitachi, F 1050).

For monoamines separation, Inertsil 3 ODS-3 $100 \times 4.6 \times 3$, Chrompack column was applied. Mobile phase consisted of: 94 mM sodium phosphate monobasic, 0.6 mM 1-octanesulfonic acid sodium salt, 1.36 mM EDTA, 1.43 M acetonitrile, pH 4.05. Column temperature during separation was maintained at 35°C. For detection of separated monoamines, amperometric detector was used (Merck-Hitachi, LaChrom, L-3500A). Samples of 20 μl were injected manually.

Chemicals
All chemicals of HPLC grade were from Sigma (Germany) except for sodium phosphate, sodium acetate and solvents, which were purchased from POCh (Poland), and 1-octanesulfonic acid sodium salt was purchased from Fluka (Germany). Standards were purchased from Sigma (Germany).

Data analysis
The chromatographic data were collected and processed using Chromatography Data Station Software (version 3.1.1, Merck-Hitachi Model D-7000).

The Wilcoxon test for matched pairs and sign test for related samples were applied.[15] Pair consisted of samples of corresponding spinal cord tissue of exercised and nonexercised rats, which were analyzed consecutively.

Results

Changes of segmental distribution of amino acids: Glu, Asp, Gly and GABA in the spinal cord owing to long-lasting locomotor exercise
Among all tested neurotransmitters Glu homogenate concentration was the highest in all analyzed spinal segments. In thoracic (T) and rostral lumbar (L1/2) segments of non-exercised animals, its concentration fluctuated around 2150 pmol/mg wt (400 ng/mg of wt) and increased caudally reaching over 2800 pmol/mg wt (500 ng/mg of wt) in sacral (S) segments (Figure 1). The sign test was applied to verify whether the concentration of Glu was distributed in the spinal cord according to rostro-caudal gradient (ie, that $T<L1/2<L3–5<S$). This analysis revealed that intersegmental rostro-caudal gradient of Glu homogenate concentration appeared in majority of animals (Table 1). Only the differences between L3–5 and L1/2 were significant ($P<0.02$). In L3–5 segments, a mean Glu concentration was higher by about 11% than in L1/2 segments (Wilcoxon, $P<0.04$).

Locomotor exercise caused minor intersegmental fluctuations of Glu homogenate concentration, but modified its rostro-caudal gradient (Figures 1 and 2, Table 1). In particular, in majority of exercised animals the Glu level in rostral and caudal lumbar segments changed in an opposite direction. Mean Glu homogenate concentration in L1/2 segments of exercised animals was about 5% higher, whereas in caudal lumbar segments it was about 7% lower than in nonexercised animals (Figure 2). Both in thoracic and sacral segments, the changes of Glu concentration were negligible (Figures 1 and 2). None of these differences reached statistical significance.

Asp homogenate concentration in nonexercised rats was about 2 times lower than that of Glu (Figure 1). In lumbar segments of nonexercised animals, it was about 1050 pmol/mg wt (140 ng/mg of wt), but it increased progressing towards the sacral segments where it reached about 1430 pmol/mg wt (200 ng/mg of wt) (Figure 1). The sign test (Table 1) confirmed that the concentration of Asp was also distributed in the spinal cord according to rostro-caudal gradient, that is, $T<L1/2<L3–5<S$ ($P<0.03$, $P<0.02$, $P<0.01$, respectively). In L3–5 segments a mean Asp concentration was on the average higher by 10% than in L1/2 segments.

Following locomotor exercise, segmental homogenate concentration of Asp also changed so that the rostro-caudal gradient became insignificant (Table 1). The most pronounced change appeared in the lumbar segments where in three out of seven exercised animals, the Asp level in rostral and caudal lumbar segments changed in the opposite direction. Figure 1 shows that the differences between the Asp concentration in the

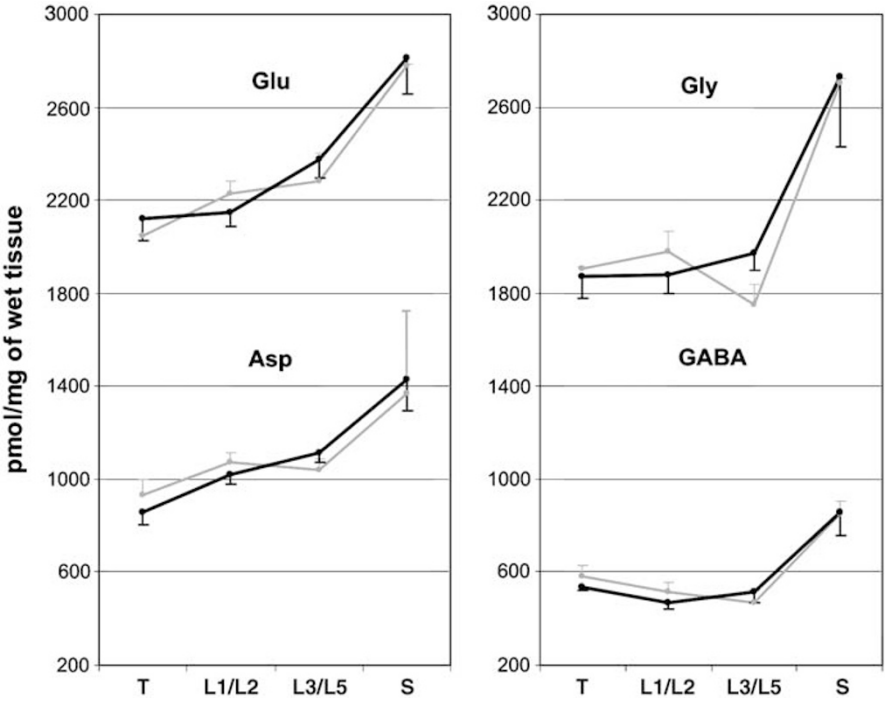

Figure 1 Glu, Asp, Gly and GABA concentrations in the mid- and caudal thoracic (T), rostral lumbar (L1/2), caudal lumbar (L3–5) and sacral (S) segments in nonexercised (black) and exercised (gray) rats. The data are expressed as the mean ± SEM

Table 1 The results of Sign test verifying rostro-caudal gradient of amino acids concentration in the spinal cord of non-exercised and exercised rats[16]

Amino acid	*Segmental comparison*	*Non-exercised*			*After locomotor training*		
		N	x	P	N	x	P
Asp	T<L1/2	5	0	<0.03	4	0	NS
	L1/2<L3–5	6	0	<0.02	7	3	NS
	L3-5<S	7	0	<0.01	5	1	NS
Glu	T<L1/2	5	1	NS	4	1	NS
	L1/2<L3–5	6	0	<0.02	7	4	NS
	L3-5<S	7	2	NS	5	2	NS
Gly	T<L1/2	5	0	<0.03	4	1	NS
	L1/2<L3–5	6	0	<0.02	7	6	NS
	L3-5<S	7	1	NS	5	0	<0.03
GABA	T<L1/2	5	2	NS	4	2	NS
	L1/2<L3–5	6	1	NS	7	5	NS
	L3-5<S	7	1	NS	5	0	<0.03

N, the total number of compared pairs; x, the number of pairs not fulfilling the prediction
Spinal cord segments: T, mid- and low thoracic; L, lumbar, S, sacral; NS, non significant

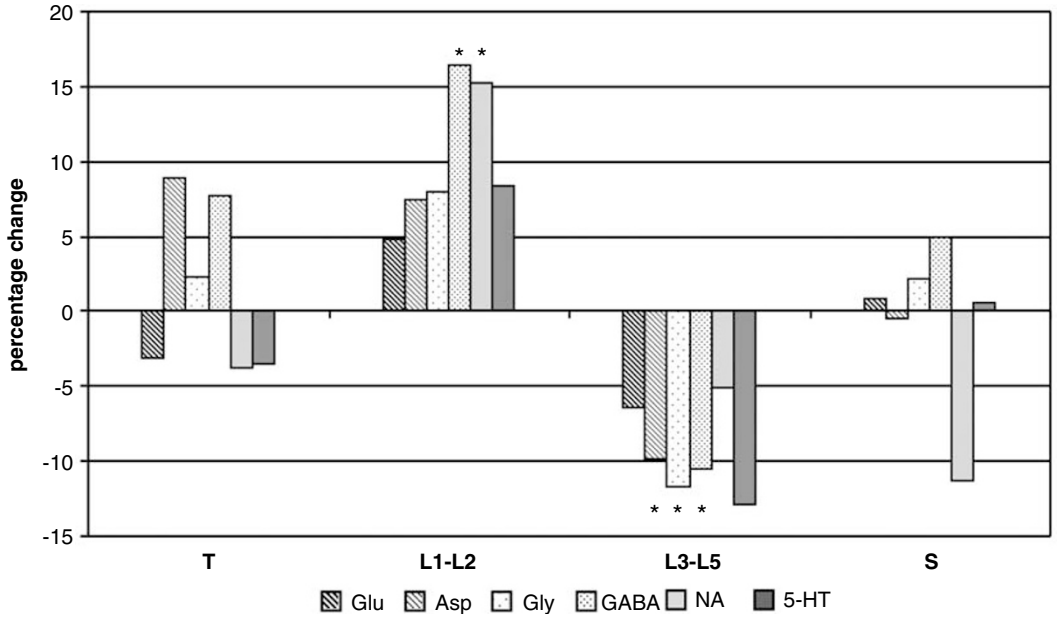

Figure 2 Effect of long-lasting, moderate treadmill walking on the concentrations of amino acids (Glu, Asp, Gly, GABA) and monoamines (NA and 5-HT) in the spinal cord of the rat. The results are expressed as percent of corresponding control and are the mean of all tested animals. Stars indicate statistical significance of differences (ranging from $P<0.02$ to $P<0.04$) between nonexercised and exercised animals

rostral and caudal lumbar segments flattened. This change was caused by a 10% decrease of Asp concentration in the caudal lumbar segments ($P<0.02$) and the concomitant, opposite tendency in the rostral lumbar segments (Figures 1 and 2).

Gly homogenate concentration in the spinal cord was comparable to that of Glu and was about 1870 pmol/mg wt (140 ng/mg wt) in the thoracic segments of non-exercised animals (Figure 1). Also Gly concentration showed a rostro-caudal gradient progressing towards the sacral segments where it reached about 2750 pmol/mg wt (over 200 ng/mg wt) (Figure 1). The sign test confirmed that the concentration of Gly was also distributed in the spinal cord according to rostro-caudal

gradient, which was significant for T *versus* L1/2 and L1/2 *versus* L3–5 ($P<0.02$) (Table 1). The mean Gly concentration was higher by about 5% in L3–5 than in L1/2 segments (Figure 1).

Again, the rostro-caudal gradient of Gly homogenate concentration was disturbed after locomotor exercise as the changes, which appeared in the rostral and caudal lumbar segments, were opposite (Figure 1). In six out of seven exercised animals, Gly homogenate concentration in rostral lumbar segments was higher than in caudal ones. Mean Gly concentration was reduced owing to exercise by about 13% (Wilcoxon, $P<0.02$) in the caudal lumbar segments (L3–5) (Figure 2). The opposite tendency appeared in rostral lumbar segments, but it did not reach statistical significance (Figure 2). The effect of exercise on the Gly concentration in the thoracic and sacral segments was negligible (Figure 2).

GABA homogenate concentration was relatively low in the spinal cord. In thoracic segments of nonexercised animals, it reached about 540 pmol/mg wt (about 56 ng/mg wt) (Figure 1). In rostral lumbar segments its concentration was lower by about 14% than in thoracic and caudal lumbar segments (Figure 1). In sacral segments, it reached 860 pmol/mg wt (about 90 ng/mg wt) (Figure 1). However, the rostro-caudal gradient of GABA concentration (T<L1/2<L3–5<S) was not significant.

The exercise caused changes of GABA homogenate concentration similar to those of other amino acids. Thus, GABA concentration increased by about 17% in L1/2 segments (Wilcoxon, $P<0.03$) and decreased by about 11% in L3–5 segments (Wilcoxon, $P<0.02$). Exercise exerted minor effect on GABA concentration both in thoracic and in sacral segments (Figure 2).

Changes of segmental distribution of monoamines in the spinal cord owing to long-lasting locomotor exercise

In nonexercised animals, mean homogenate concentrations of 5-HT and NA in the thoracic segments were approximately 1.2 and 1.7 pmol/mg wt, respectively (about 0.3 ng/mg wt) and they increased progressing towards the sacral segments, particularly for 5-HT level (Figure 3). This tendency (ie, that T<L1/2<L3–5<S) did not reach statistical significance (Table 1). The mean concentration of 5-HT in the lumbar and sacral segments was over twice as high as that found in the thoracic segments (Figure 3). The 5-HT concentration in the L3–5 segments was about 19% higher than in L1/2 segments. Segmental homogenate concentration of 5-HIAA also followed the rostro-caudal gradient (Figure 3).

Similar to 5-HT but less pronounced differences appeared in the NA homogenate concentration, which was approximately 8% lower in L1/2 than in L3–5 segments.

Dopamine concentration measured in the whole tissue homogenate was generally very low (about 0.3 pmol/mg wt) (0.06 ng/mg wt), thus intersegmental variations were not analyzed.

Long-lasting, moderate locomotor exercise modulated segmental distribution of 5-HT and NA homogenate concentration (Figures 2 and 3). It attenuated 5-HT concentration in caudal lumbar segments by about 13% (Figure 2). An opposite tendency was observed in rostral lumbar segments where 5-HT concentration was found to be higher by about 8% than in nonexercised animals, but none of these differences reached statistical significance (Figure 2). The effect of exercise on 5-HT homogenate concentration in thoracic and sacral

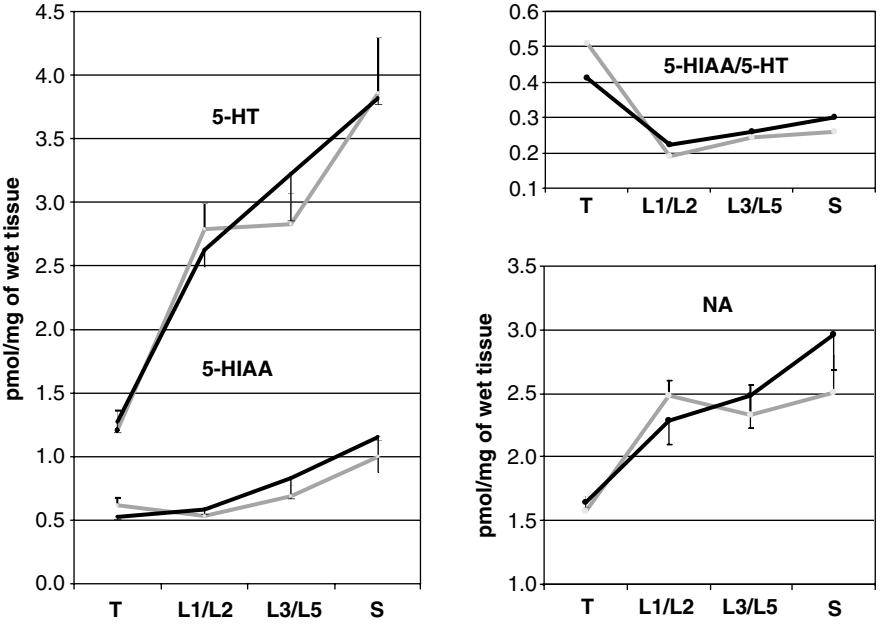

Figure 3 NA, 5-HT and 5-hydroxyindoloacetic acid (5-HIAA) concentrations and the ratio of 5-HIAA to 5-HT in the mid- and caudal thoracic (T), rostral lumbar (L1/2), caudal lumbar (L3–5) and sacral (S) segments in nonexercised (black) and exercised (gray) rats. The data are expressed as the mean ± SEM

segments was negligible (Figure 2). The training affected 5-HT metabolism by increasing 5-HIAA/5-HT ratio in T segments and revealing a tendency to reduce it in other segments (Figure 3). As the 5-HT level in thoracic segment was maintained at control levels, metabolized 5-HT pool was most probably supplemented with newly synthesized/ transported 5-HT.

Noradrenaline homogenate concentration decreased in thoracic, caudal lumbar and sacral segments owing to exercise (Figures 2 and 3). Again, an opposite tendency was observed in rostral lumbar (L1/2) segments, where NA homogenate concentration increased by about 15% following exercise ($P<0.05$) (Figure 2).

Discussion

Segmental distribution of amino acids and monoamines in the spinal cord of nonexercised rats

Our results on neuroactive amino-acid content in the spinal cord of nonexercised animals have revealed that Glu and Gly were present in the highest amounts in all tested segments. There was over a three-fold ratio of Gly to GABA content and a two-fold ratio of Glu to Asp content. These results are in line with earlier studies and confirm observation suggestive of a predominant role of Glu and Gly in spinal transmission.[17,18] One should be aware that the approach taken in this study (ie, of amino-acid measurements in the whole tissue homogenate) limits drawing conclusions pertaining amino-acidergic transmission, because neurotransmitter pools of these compounds represent only a fraction of their total tissue content. However, the results allow concluding on a neurochemical modeling involving amino acid metabolism. The discussion below is based on the premise that regionalized distribution of amino acids, which are used for a variety of metabolic functions, could indicate that their neurotransmission-related role in regions where they are present in relatively higher amount is prominent.

Comparison of our results obtained on spinal segments taken in *toto* with those limited to spinal gray analysis [16] shows even higher Glu/Asp and Gly/GABA ratios for the latter. This is indicative of different amino-acid content in spinal white matter as compared to gray matter that may lead to more pronounced differences when only gray matter is taken into account. Irrespective of the absolute values of amino-acid content found in our study, there was a clear rostro-caudal gradient of their concentration in the whole tissue homogenate of the caudal thoracic, lumbar and sacral segments of the spinal cord. Our data enrich available results disclosing the differences in the content of amino acids and monoamines between the rostral and caudal lumbar segments, not reported earlier. It is worth mentioning that the ratio between the area covered by grey and white matter, which is also progressing caudally (Th = 0.5; L1/2 = 0.7; L3–5 = 1.0; S = 2.5), might only partly account for the gradient.[19] For instance, the ratio of gray to white matter in L3–5 is twice as high as in low thoracic segments, but the GABA concentration there were nearly the same (Figure 1). Similarly, segmental changes of 5-HT and NA concentrations only partly correspond to changes of these ratios (Figure 3).

Generally, the monoamine homogenate concentration in the spinal cord was two orders lower than that of amino acids. The level of dopamine was the lowest among all tested monoamines in agreement with earlier observations.[20,21] The 5-HT and NA levels were comparable. The highest 5-HIAA/5-HT ratios in our experiments and the lowest 5-HT levels in thoracic segments may suggest that 5-HT turnover in the resting state was the highest in these segments. Our observations on the rostro-caudal gradient of 5-HT, 5-HIAA and NA confirm earlier data obtained for the rats[20–22] and for the cats.[23]

Although there is no clear correlation between distribution of monoamines and their receptors along the spinal cord,[24–27] there are indications that the density of 5-HT_7 receptor, which is implicated in the control of locomotion, is higher in the rostral than in caudal lumbar segments.[26–28] Recently, the data obtained in isolated neonatal mouse spinal cord preparations by Christie and Whelan[29] revealed that combinations of monoaminergic compounds established a rostro-caudal organization of locomotor rhythms. This links the neurochemical and functional rostro-caudal gradients.

Long-lasting locomotor exercise modulates the rostro-caudal gradient of amino acids and monoamines distribution

The most striking observation in this study was that a long-lasting, moderate locomotor exercise distorted a rostro-caudal gradient of amino-acid and monoamine concentration in the whole tissue homogenate of the caudal thoracic, lumbar and sacral segments of the spinal cord. It was mainly due to an opposite effect of exercise exerted on their concentration in the rostral (L1/2) and caudal (L3–5) lumbar segments. In the rostral lumbar segments, the homogenate concentration of all tested compounds showed a tendency to increase, whereas in the caudal lumbar segments, it was reduced owing to exercise. Thus, the differences induced by long-lasting, moderate locomotor exercise revealed neurochemical diversity of these two parts of the lumbar spinal cord involved in the control of locomotion, otherwise not detected.

The effect of locomotion was tested shortly after the last session of the exercise has been completed. Thus the observed decrease of amino acids and monoamines concentration in the caudal lumbar segments might be prescribed to higher rates of release, turnover and clearance of these compounds. However, the opposite effect of the exercise on neuroactive compounds in the homogenates from the rostral lumbar segments does not favor this interpretation.

Numerous data obtained in spinalized cats confirm the importance of the interneuronal network located rostrally in the segments, where motoneurons innervat-

ing hindlimb muscles are located in the expression of spinal locomotion;[30] for a review see Rossignol *et al*.[4] Similarly, Ribotta *et al*[31] delivered the evidence stressing an importance of rostral lumbar segments in the control of locomotion of the rat. They showed that the grafts of embryonic mesencephalic cells below the spinal lesion site (at caudal thoracic segments) improved locomotor function only if 5-HT reinnervation from the graft reached L2 segment of the spinal cord in the rat.[31]

Studies of neonatal rat spinal cord *in vitro* also stressed the crucial role of interneuronal network at L1 and L2 segments in driving hindlimb locomotion.[10,32] Several other lines of evidence indicate that in the neonatal rat, the network of interconnected spinal interneurons, which are capable of generating locomotor-like activity, located in the rostral lumbar segments shows greater capacity for rhythmicity than that in other segments.[13,14,28] Noteworthy, Gabbay and Lev-Tov[33] showed that in early developmental period not only rostral lumbar but also sacrococcygeal segments responded differently to NA than to caudal lumbar segments. The bath-applied NA induced fast rhythms, which mimicked locomotor activity of the spinal cord in rostro-lumbar and sacrococcygeal, but not in caudal lumbar segments.[33]

All these data point to segmentally differentiated capability of spinal networks in generation of locomotor activity, but they also stress an importance of the network in the rostral lumbar segments in the control of locomotion. Our results speak in favor of the latter as long-lasting, moderate locomotor exercise allowed revealing the neurochemical differences between these two parts of the lumbar spinal cord in the adult rat, which may reflect different regulatory mechanisms of the enzymes, which maintain the appropriate levels of neurotransmitters for a long-term function.

Majority of data on the effect of physical exercise on the modulation of the neurotransmitter concentration in the mature spinal cord are limited to the caudal lumbar segments. Generally, our results are in line with these observations. Glu measured in extracellular space at the L5 spinal segment of the rat by means of microdialysis probe increased dynamically during locomotion but returned to resting levels already 40 min later.[6] Similarly, we have observed that Glu level measured in the whole tissue homogenate of caudal lumbar segments, 2 h after the termination of the last locomotor session was close to that of control animals. It is worth mentioning that the effect of training on Asp was stronger than that on Glu, the difference being prominent in caudal lumbar segments (Figure 2). As in lumbar segments, Asp has been postulated to be associated predominantly with local interneurons, the effect on Asp may be used as evidence for suggesting a predominant influence of training on this pool of spinal neurons.[17] With all the reservations towards considering Asp as a neurotransmitter, there is evidence on neuromodulatory function of Asp in the spinal cord, which, as we show, may be modulated by long-term locomotor training.

Several groups have assessed the effect of locomotor training on the modulation of GABA and Gly in the spinal cord.[5,7,34,35] The administration of strychnine, a glycinergic antagonist, improved stepping in spinalized animals.[5] Also downregulation of GABA synthesis was shown to be inversely correlated with the stepping ability.[7] The authors attributed beneficial effect of training on the stepping ability in spinal cats to attenuation of GABA and Gly synthesis in the segments caudal to the lesion. Our findings showing a significant decrease of GABA and Gly concentration in the whole tissue homogenate of the lumbar segments L3–5 following locomotor exercise are very much in line with these observations. However, we have found this effect confined to caudal lumbar segments. In other segments, in particular, in rostral lumbar segments, the tendency was opposite.

Involvement of descending serotonergic, noradrenergic and dopaminergic pathways to the spinal cord in the control of locomotion through the modulation of motoneuron activity has been well documented.[4,26] The effect of locomotor exercise on the concentration of biogenic amines in the spinal cord was assessed by several groups.[8,9,36] As shown by Gerin *et al*,[8] MHPG, a NA metabolite, DA and 5-HT concentration measured by microdialysis probe in the ventral funiculus at L4 segments increased during locomotor exercise and decreased during rest. Surprisingly, the concentration of 5-HT in dialysates of the ventral horn at L4, collected during exercise, was decreased by 9% compared to the mean value during rest. It decreased more (by 31%) when measured 1.5 h after exercise.[9] 5-HIAA also decreased in a similar way as 5-HT. On the other hand, MHPG concentration slightly increased during exercise and decreased thereafter. We have observed similar tendency of diminishing of both 5-HT and NA concentration in the whole tissue homogenate of caudal lumbar segments 2 h after termination of locomotor exercise. However, again, this effect was confined to the caudal lumbar segments, and in the rostral lumbar segments, the tendency was opposite.

Conclusions

Taken together, our results indicate that moderate, long-lasting locomotor exercise leads to regionally differentiated neurochemical remodeling in the rat lumbar spinal cord. This phenomenon suggests different regulatory mechanisms in the rostral and caudal lumbar segments. Our observation poses the question whether different neurochemical responses in the rostral and caudal lumbar segments are related to a leading role of rostral lumbar segments in distributed organization of the spinal locomotor network, the concept discussed recently by Langlet *et al*.[37]

Interestingly, long-lasting locomotor exercise causes also an increase of brain-derived neurotrophic factor (BDNF) level in the spinal cord.[38] It has been well documented that BDNF may act as a modulator of synaptic transmission in the spinal cord.[39–41] It is thus

possible that interactions between BDNF and neurotransmitters' release owing to exercise (eg with 5-HT, Glu, GABA) might give us a cue for understanding a functional plasticity in the spinal cord.

Further studies on the kinetics and expression of amino-acid metabolizing enzymes, their transporters and receptors should reveal whether the changes in the total amino-acid contents in the spinal tissue found in this study are related to changes in neurotransmission.

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