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Extracellular Glutamate in the Dorsal Horn of the Lumbar Spinal Cord in the Freely Moving Rat During Hindlimb Stepping

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WALWYN, W. M., J. TA-HUANG, L. ACKERSON, N. T. MAIDMENT AND V. R. EDGERTON. *Extracellular glutamate in the dorsal horn of the lumbar spinal cord in the freely moving rat during hindlimb stepping.* PHAMACOL BIOCHEM BEHAV **63**(4) 581–588, 1999.—The capacity to reestablish locomotor function after complete spinal cord transection in the adult mammal is now well documented. Further studies have shown different neurotransmitters to be involved in the initiation and maintenance of these locomotor patterns. However, there has been no in vivo evidence of the changes in glutamate or any other neurotransmitter in the extracellular space of the dorsal horn during an alternating motor pattern such as hindlimb stepping. This study describes an in vivo microdialysis technique to measure extracellular glutamate in the dorsal horn of the spinal cord in the fully awake intact rat. A concentric microdialysis probe was placed in the dorsal horn at L5, and 18 h later dialysate samples were collected at 20-min intervals before, during, and after 20 min of hindlimb stepping. During stepping, extracellular glutamate rose 150% above resting levels and returned to resting levels 40 min later. This increase may have occurred either as a result of primary afferent depolarization or modulation by the descending and ascending supraspinal pathways. In another series of experiments extracellular glutamate was, therefore, measured in the dorsal horn of the chronic spinally transected rat during 20 min of hindlimb stepping. Although the spinal group did not take as many steps as the intact group, those taking more than 40 steps showed a significant rise in extracellular glutamate, and the number of steps taken by the individual spinal rats correlated positively with the individual values of extracellular glutamate ($r^2 = 0.63$). These results are consistent with glutamate being an important neurotransmitter in the spinal cord in normal locomotion. © 1999 Elsevier Science Inc.

Glutamate Locomotion Microdialysis Spinal cord Spinal transection

THE capacity to reestablish locomotor function after complete spinal cord transection in the adult mammal is now well-documented (3,35). Further ability of the spinal cord to integrate and execute complex motor patterns in the absence of supraspinal influence in acute and chronic spinally transected animals may be altered by the application of various pharmacological agents targeting different receptors (4). These studies, as well as many of fictive locomotor patterns, suggest that hindlimb locomotion results from a complex interplay of different neurotransmitters and neuromodulators within the spinal cord. Although there has been no in vivo information regarding glutamatergic transmission during alternating locomotor patterns of the left and right hindlimbs, or hindlimb stepping, in vitro studies indicate that glutamate and the glutamatergic receptors are involved in the rhythmic properties of hindlimb locomotion. When applied to an isolated neonatal spinal cord in situ, L-glutamate and agonists of the glutamatergic receptors induce fictive locomotion in the neonatal rat (1,10,14,32), chick (2), and lamprey (13,31).

Glutamate, an excitatory amino acid, is found extensively throughout the mammalian spinal cord. It is found in neurons of the dorsal horn and dorsal root ganglia (5,66), stored in nociceptive and nonnociceptive primary afferent terminals (8), interneurons (42), and motoneurons (9,41). A number of de-

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scending and ascending pathways are glutamatergic (22) and release glutamate in the spinal cord following stimulation of the raphe magnus and areas of the cortex in anesthetized animals (17,52,62). Glutamate and the ionotrophic and metabotropic glutamatergic receptors are instrumental in relaying a broad range of information within the spinal cord. Noxious stimuli such as intradermal formalin (37,60) and capsaicin (58,61) increase extracellular glutamate in the dorsal horn in vivo. Stimulation of the high threshold C fibers and Aδ fibers and the low threshold Aβ fibers release glutamate in vitro (27–29,65). Other stimuli, such as sustained muscle contraction, increase extracellular glutamate in the dorsal horn; this increase may be prevented by a dorsal rhizotomy of the L7 afferents (23), suggesting that glutamate is released by the primary afferent terminals. However, CNQX prevents a rise in glutamate following stimulation of the sciatic nerve at C-fiber strength showing that glutamate is released by the interneurons rather than primary afferent terminals, at least in this experimental model (45). The release of glutamate in the dorsal horn is not restricted to these stimuli, traumatic incidents such as acute spinal cord injury (33), spinal cord ischemia (53), and acute inflammation (59) increase extracellular glutamate in the dorsal horn of the spinal cord.

The contribution of the excitatory amino acids to increased transduction sensitivity of the primary afferent nociceptors, or peripheral sensitization (50), and enhanced excitability of the spinal cord dorsal horn neurons, or central sensitization (67) has been extensively studied (12). The resulting state of hyperalgesia may be mimicked by the application of glutamate (21) and is blocked by antagonists of the NMDA receptors (64). This sensitization is marked by an increased responsiveness of the dorsal horn neurons to the excitatory amino acids (15) and by changes in the receptive fields of these neurons (67).

Glutamate and the glutamatergic receptors are, therefore, involved in the transmission of different types of stimuli in the spinal cord, ranging from noxious chemical, mechanical, and thermal stimuli to stimuli relaying proprioceptive information and the modulation of sensory information. However, the role that extracellular glutamate may play during an acquired complex but repetitive movement such as hindlimb stepping is unknown.

Using in vivo microdialysis we asked whether hindlimb stepping is accompanied by a change in extracellular glutamate in the dorsal horn of the spinal cord. If extracellular glutamate is involved in relaying information during hindlimb stepping, this may result from altered glutamate release and reuptake in the local neuronal circuitry of the spinal cord in addition to modulation by the descending and ascending supraspinal pathways. In the following experiment we determined how removal of the ascending and descending supraspinal pathways by spinal transection affects the extracellular amino acids of the spinal cord, and whether hindlimb stepping modulates these amino acids equally in the chronic spinal and intact rat.

METHOD

Female Sprague–Dawley rats (150–175 g) were randomly assigned to two experimental groups, one of which was not spinally transected (Experiment A: the intact group, $n = 16$). The second group was spinally transected at the midthoracic level (Experiment B: the spinal group, $n = 16$). Both groups were kept in the same housing and lighting conditions for the duration of the experiment.

Experiment A

For the 7 days prior to the microdialysis experiment the intact group walked on the treadmill at 0.1 $m \cdot s^{-1}$ for 10-min periods, a speed equivalent to a slow walking pace in adult rats. So as to minimize the surgical intervention immediately prior to the microdialysis experiment a guide cannula was first implanted, and 7 days later the microdialysis probe was gently lowered through the guide cannula into the spinal cord. We found that implanting a guide cannula and then the microdialysis probe resulted in lower and more stable levels of glutamate within the dialysate.

While under halothane anesthesia and sterile surgical conditions a premeasured guide cannula was stereotaxically implanted 1.5 mm from the midline, at an angle of 15° from the vertical and 1.5 mm into the vertebral arch of T13. This placed the cannula at the anterior surface of the vertebral bone so as not to penetrate the dural sheath. The guide cannula was sealed and then fixed in place by three screws and dental cement; one screw was placed in the contralateral vertebral arch of T13, and two in the vertebral arch of T12. Dental cement was placed around the screws so as to form a solid arthrodesis between the two vertebra and then leveled to the height of the guide cannula. The incision was sutured closed.

Seven days later a microdialysis probe, of concentric design (68) and membrane length of 1.5 mm, was lowered into the guide cannula so that 1.5 mm of the probe or the membrane of the probe, extended below the end of the guide cannula into the dorsal horn (Fig 1). So as to mininize the length of active membrane placed out with the dorsal horn, the terminal 0.25 and proximal 0.25–0.5 mm were made inactive during the construction of these probes. This results in 0.5 mm of the membrane within the dorsal horn of the spinal cord and 0.5–0.25 mm within the subvertebral space. The probe was cemented in place and the skin sutured loosely around the probe. The microdialysis tubing was loosely tethered to a collar placed around the animal's neck and attached to a single channel swivel mounted on a cantilever arm. This method fixes the probe onto the vertebra and eliminates movement within the vertebral space.

Throughout the experiment the probe, perfused with artificial CSF (aCSF), was kept at a flow rate of 0.5 $\mu l \cdot min^{-1}$. Eighteen hours later baseline samples were collected at 20-min intervals for 60 min while in the holding cage, during the 20 min of being placed on the moving treadmill belt and for a further 60 min once returned to the holding cage. While on the treadmill the intact rats walked freely, and the stepping patterns were recorded and analyzed for the number of steps taken during the first 5 min of the 20-min hindlimb stepping period. Upon completion of the experiment, the spinal cords were removed, frozen, and examined for placement of the probe by cresyl violet staining.

Experiment B

In this experiment the microdialysis experiment followed the same protocol. However, these animals were initially transected at the midthoracic level and then trained or not trained to step. This group underwent the following protocol of spinal transection while under aseptic surgical conditions; and an anesthetic of 70 $mg \cdot kg^{-1}$ ketamine hydrochloride and 5 $mg \cdot kg^{-1}$ acepromazine maleate was delivered intraperitoneally. A skin incision was made over the midthoracic area, the dorsal fat pad folded rostrally, and the paraspinal muscles parted using blunt dissection techniques. A small laminectomy exposed the dura, which was then lifted away from the

spinal cord and cut. Following 2–3 drops of topical lignocaine hydrochloride (2%), the spinal cord was transected at a single site using microdissection scissors, taking care to leave the ventral artery intact. The transection was considered complete when the two ends of the spinal cord separated spontaneously by 2–3 mm. This space was filled with gelfoam, the paraspinal muscles and fat pad sutured in place using internal sutures, and the overlying skin sutured. The animals recovered in an incubator until wakening, and were then transferred to cages and kept at an ambient temperature of 25°C for 5 days. A postsurgical prophylactic antibiotic, Baytril, was administered intramuscularly (0.2 ml) twice daily for 48 h. Bladders were manually evacuated three times daily for the first 7 days, twice daily for the following 7 days, and then once daily for the remainder of the experimental period. Once the skin incision had healed, animals were housed in pairs and examined daily for signs of adequate hydration and defecation, urinary tract infection, skin lesions, and weight loss.

Half of the spinally transected group, the spinal trained group, were trained to step on a treadmill at 0.1 $m \cdot s^{-1}$ for 10 min a day, 5 days a week, for 9 weeks. The front paws of the trained spinal rats rested on a Perspex plate placed over the front of the treadmill and a divider placed between the hindlimbs to ensure that the hindlimbs did not cross the midline. During each training session specific sensory stimuli were applied to facilitate alternating stepping patterns of the hindlimbs. These stimuli included placing of each paw alternately, applying light tactile stimulation to the skin over the knee and abdomen, and increasing the load carried by the hindlimbs by applying pressure through the tail. The duration and pressure of each stimulus was standardized across all animals, and noxious stimuli were avoided. Progress was assessed by a weekly 10-min kinematic videorecording of the trained and untrained spinally transected groups during which no stimulus was applied. For the 7 days prior to the final microdialysis experiment the spinal nontrained rats were placed on the treadmill for 10-min periods so as to habituate to the treadmill environment.

The in vivo microdialysis experiment followed the same protocol as described for Experiment A, but while on the treadmill the spinal animals were supported under the upper torso with the weight-bearing hindlimbs on the treadmill belt, no sensory stimulus was applied. The stepping patterns were recorded and analyzed as described for Experiment A.

Chromatographic Analysis

Dialysate amino acid content was analyzed by high-performance liquid chromatography (HPLC) with fluorometric detection. Amino acids were derivatized with *O*-pthaldehyde (OPA) prior to automated injection into the HPLC apparatus. The OPA derivatizing agent was prepared by adding 40 μl of OPA 100 $mg \cdot ml^{-1}$ of methanol), 5 μl of β-mercaptoethanol to 3.95 ml of 0.125 M boric acid buffer (pH 10). A 30-μl aliquot of OPA reagent was reacted with the sample for 30 s. The OPA–amino acids adducts were resolved on a reverse-phase 3 × 150-mm column (Hypersil, 3 μm, C18, Keystone Scientific) with sodium acetate (35 mM, pH 5.9 with glacial acetic acid) 1% tetrahydrofuran, and 0.01% v/v TEA as the aqueous solvent. The organic mobile phase consisted of 70% acetonitrile, 15% methanol, and 15% sodium acetate (35 mM final concentration), pH 7.65 with glacial acetic acid. The flow rate was 0.6 $ml \cdot min^{-1}$ with the gradient profile set so as to increase the organic mobile phase from 10.5 to 15% in 3 min, to hold this percentage at 15% for the next 3 min, and then to further increase it from 15 to 33% in the following 9 min. The column was then washed with a gradient of 33 to 65% of organic mobile phase in 0.5 min, held at 65% for 2 min and finally returned to 10.5% in 0.5 min. The column was equilibrated at 10.5% for 5 min before injection of the next sample. Complete analysis required 24 min. The limit of detection was 5 fmol, and the amount of each measured amino acid in the sample was quantified using an external standard. This whole procedure, including data collection and calculations was automated using Gilson hardware and software.

Statistical Analysis

Data from experiments in which the flow rate varied or where the microdialysis probe was found to be located outside of the dorsal horn were excluded from the analysis. Samples were collected before, during and after, hindlimb stepping. Amino acid levels of each animal were normalized to the mean of three resting samples taken during normal cage activity immediately prior to hindlimb stepping, and presented as a percentage of this basal value. Data were analyzed using the nonparametric Kruskal–Wallis test; statistical significance was accepted at $p < 0.05$ and data presented as mean ± SEM.

RESULTS

Experiment A

The dialysate content of glutamate in the spinal cord during normal cage activity (mean of three resting samples) measured in the intact rats was 2.03 ± 0.60 $\mu mol \cdot l^{-1}$. During the 20 min of walking on the treadmill dialysate glutamate content rose 150% above resting levels ($p < 0.05$) to 3.32 ± 1.08 $\mu mol \cdot l^{-1}$, and returned to resting levels within 40 min of being placed in the home cage (Fig. 2a) Dialysate aspartate content showed a similar trend during hindlimb stepping, but was not significant (Fig. 2a). The dialysate content of a nonneurotransmitter amino acid, histidine, did not change during or after the period of hindlimb stepping (Fig. 2a) indicating some level of specificity of the glutamate response.

Experiment B

After 5 weeks of being trained to step, the spinal trained group took more steps than the spinal untrained group during 10 min of unstimulated, unassisted stepping. During the final experiment the trained and untrained groups took the same number of weight-supporting steps, and both groups took significantly fewer steps than the intact group during this period (spinal; 39 ± 19 and intact; 450 ± 15 weight-supporting, unassisted steps during the first 5 min of stepping). However, some of the spinal group took more than 40 steps (10% of the number of steps taken in the intact group) during the microdialysis experiment, and others less than 40 during this period. Data from the spinal trained and untrained groups has, therefore, been consolidated, initially presented as one group, the spinal group, and then divided into two groups, those taking more and those taking less than 40 steps during the microdialysis experiment.

Basal dialysate glutamate was higher in the spinal than intact group (Table 1) and did not alter during hindlimb stepping (Fig. 2b). Basal dialysate aspartate content was not different between the intact and spinal groups (Table 1) and, in the spinal groups, did not change during the time of being placed on the treadmill (Fig. 2b). The dialysate content of a nonneurotransmitter amino acid such as histidine was not different between the intact and spinal groups during normal

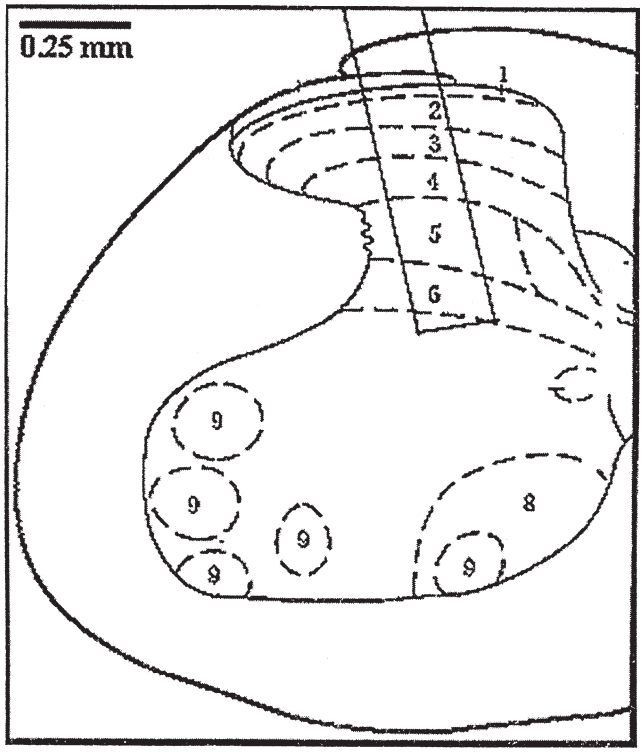

FIG. 1. A schematic drawing of L5 showing placement of the concentric microdialysis probe.

cage activity (Table 1) or as a result of being on the treadmill (Fig. 2b).

However, dialysate glutamate content (expressed as a percentage of basal values) during hindlimb stepping correlated with the number of steps taken by the spinal group ($r^2 = 0.63$, $p < 0.05$ Fig. 3). In addition, those spinal animals taking more than 40 unassisted steps during the experiment showed a significant increase in dialysate glutamate content during and immediately after stepping when compared to the resting samples (Fig. 4).

DISCUSSION

During in vivo microdialysis glutamate diffuses across the dialysis membrane from the extracellular space to the aCSF within the microdialysis probe. The results of this experiment are, therefore, presented as the content of glutamate in each 10-μl dialysate sample and are interpreted as being representative of the extracellular space in the dorsal horn of the spinal cord. However, this interpretation is made with caution,

TABLE 1

BASAL DIALYSATE GLUTAMATE, ASPARTATE, AND HISTIDINE CONTENT μmol·l^{-1}, MEAN ± SEM) OF THE INTACT AND SPINAL GROUP

Group	Glutamate	Aspartate	Histidine
Intact ($n = 7$)	2.03 ± 0.60	0.28 ± 0.09	0.70 ± 0.10
Spinal ($n = 9$)	5.22 ± 1.12*	0.87 ± 0.31	0.53 ± 0.15

*$p < 0.05$ vs. the intact group

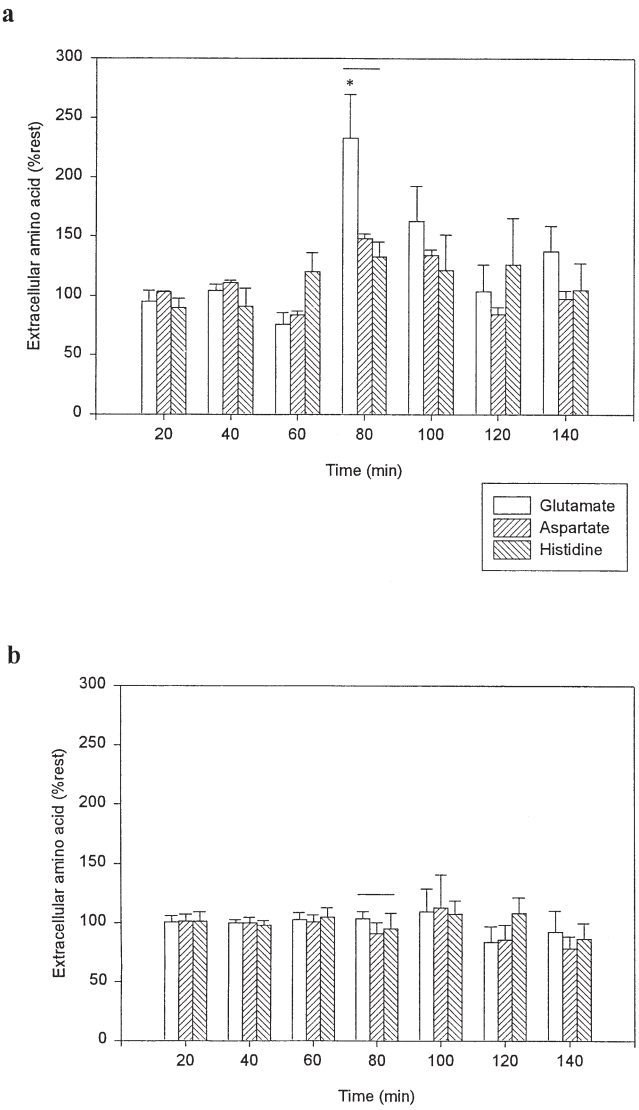

FIG. 2. Dialysate glutamate, aspartate, and histidine content (% of basal) in the dorsal horn of (a) the intact rats ($n = 7$), and (b) the spinal rats ($n = 9$) during three 20-min periods of rest (0–60 min), one 20-min period of stepping (60–80 min), and for the next three 20-min periods while the rats were resting following stepping (80–140 min).—hindlimb stepping, * $p < 0.05$ vs 20, 40, 60, 120, and 140 min of the intact group.

knowing that the microdialysis technique may only collect a small fraction of the extracellular space, and that differences in the internal and external milieu of the probe may lead to different perfusion profiles for each measured substance (46).

Different studies measuring extracellular glutamate in the spinal cord have used different types of microdialysis probes. These include transverse probes placed through the spinal cord at a specific level (61), spinal loop catheters placed within the CSF (37,38) and concentric probes placed unilaterally into a specific region of the spinal cord (19,20,23). Different animal species, experimental protocols, flow rates, and analytical biochemical protocols have also been described. The microdialysis technique described in this study is similar to that described by Gerin et al. (19,20) during which dialysis

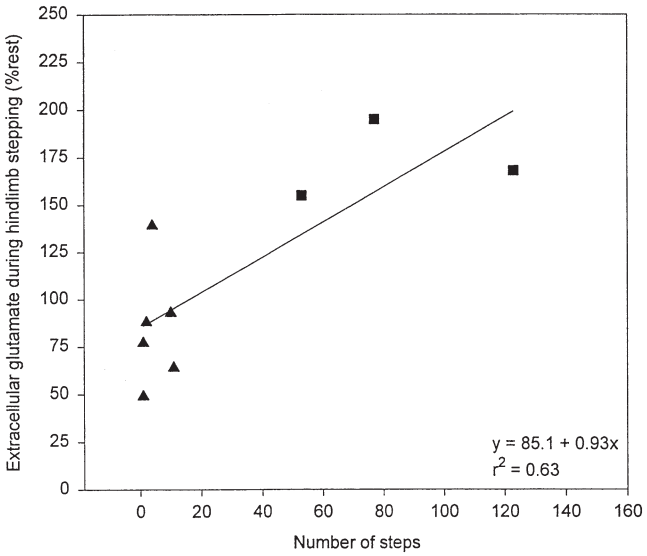

FIG. 3. The increase in dialysate glutamate content during hindlimb stepping (expressed as a percentage of basal values) correlated with the number of steps taken by the spinal ($n = 9$) rats during the first 5 min of hindlimb stepping ($p < 0.05$). ■ the spinal rats that took more than 40 steps ($n = 3$), ▲ the spinal rats that took less than 40 steps ($n = 6$) during the first 5 min of the 20 min stepping period.

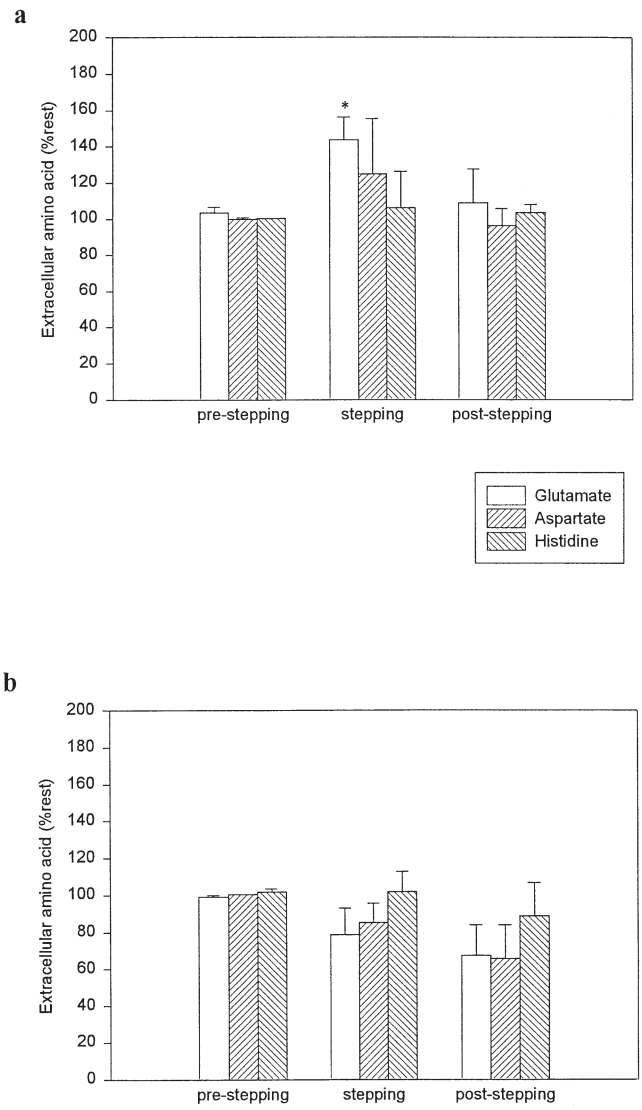

FIG. 4. Dialysate glutamate, aspartate, and histidine content (% of basal values) before, during, and after hindlimb stepping in (a) the spinal rats that took more than 40 steps ($n = 3$), and (b) the spinal rats that took less than 40 steps ($n = 6$) during the first 5 min of the 20-min stepping period. "Prestepping" refers to the mean of the three resting samples taken at 20, 40, and 60 min. "Stepping" is the mean of the samples taken at the 80- and 100-min time points, and "poststepping" is the mean of the samples taken at 120 and 140 min. *$p < 0.05$ vs. prestepping.

was performed in the freely moving animal during stepping. However, these experiments differ from this study in that the monoamines and their metabolites were measured by chronically implanted concentric microdialysis probes placed at an 18° angle from the horizontal plane into the ventral horn (20) and ventral funiculus (19). The basal dialysate concentrations of both the intact and spinal rats in this study (2–6 μM) are in the same range as previously published dialysate concentrations, i.e., 1–12 μM (33,57,61,63).

The extracellular glutamate content may be influenced by release (39) and reuptake mechanisms (24,40) of this neurotransmitter and conversion to metabolic byproducts (18). Using in vivo microdialysis Herrera-Marschitz and co-workers (24) have shown that extracellular glutamate measured in different brain regions of the anesthetized rat reflects a balance between neuronal release and reuptake within the surrounding nerve terminals and glia. Preliminary data obtained from our laboratory using the same experimental design as described in this study show that dialysate glutamate content measured in the dorsal horn of the rat increased by 78% following the perfusion of veratridine, sodium channel activator (25), and by 62% following the perfusion of L-trans-pyrrolidine-2,4-dicarboxylic acid, a selective glutamate reuptake inhibitor (7). Dialysate glutamate was also found to decrease by 30% following the perfusion of riluzole, a glutamate release inhibitor (11). (These compounds were applied to the spinal cord via the microdialysis probe.) This suggests that the dialysate glutamate content measured by in vivo microdialysis in the dorsal horn of the fully awake but resting rat reflects both the release and reuptake of this amino acid.

The microdialysis probe was placed in the dorsal horn at the segmental level of L5 monitoring the extracellular space of the dorsal laminae, an area of the spinal cord rich in afferent terminals of the lower hindlimb (47). Although the release of glutamate by the small-diameter primary afferent fibers was not well supported in the past (12), more recent studies have shown glutamate to be released in the dorsal horn by capsaicin-sensitive primary afferent fibers (27,28,65). Glutamate is also released in the dorsal horn following depolarization of low (Aβ) and high (Aδ, C) threshold cutaneous afferents (29,45,54). It is also possible that depolarization of group 1a (26,44), and group III afferents (48) leads to the release of glutamate in areas of the spinal cord.

The microdialysis membrane, some of which was present in the subvertebral space, would monitor glutamate levels within this space. Marsala et al. (38) describe a spinal loop dialysis catheter placed in the subvertebral space over the lumbar enlargement and measured resting glutamate levels of

1.7–6.1 μM. Ischemia, spinal cord compression and formalin injected into the hindpaw increased dialysate glutamate content, suggesting that these measurements taken from within the CSF do reflect activity within the local neuronal pathways.

Removal of the primary afferent fibers by dorsal rhizotomy prevents the rise in extracellular glutamate in the dorsal horn of the anesthetized cat observed during static contraction of the triceps surae muscles (23). Although not conclusive, this study suggested that glutamate was released by the primary afferent fibers during static muscle contraction. Glutamatergic interneurons in the dorsal horn (42) could account for this rise in extracellular glutamate secondary to primary afferent depolarization (45). Although the increase in extracellular glutamate in the dorsal horn of the intact rat during hindlimb stepping could have resulted from primary afferent depolarization, it could also have occurred following interneuronal and supraspinal pathway activity.

In the intact group ~450 steps were taken and extracellular glutamate rose by ~150%. Those spinal animals, with no descending or ascending pathways, who took more than 40 steps showed a rise in extracellular glutamate of ~43%, and those who took less than 40 steps showed no change in extracellular glutamate below prestepping levels. This suggests that the number of hindlimb steps, rather than the supraspinal pathways, influenced extracellular glutamate levels in the dorsal horn of the spinal cord during stepping. This could be a result of the quantity and diversity of information relayed by the primary afferent fibers from the hindlimb to the spinal cord during varying amounts of hindlimb stepping.

The number of steps and the relative increase in extracellular glutamate correlated in the individual spinal rats, which took 1–123 steps during the 20 min of being on the treadmill. The individual intact rats, which took 437–465 steps while on the treadmill, showed no such correlation, perhaps a result of the smaller range of steps taken by the intact rats or of modulatory supraspinal influences present in the intact but not spinal animals. The difference in acute sensory stimuli during the two experiments may also have accounted for the presence or absence of a correlation of extracellular glutamate with the number of steps taken in the spinal and intact groups. Conversely, chronic sensory stimulation during an intensive 9-week training period did not result in any difference in the number of steps taken or extracellular glutamate between the trained and untrained spinal groups. Of interest is that the basal levels of extracellular glutamate in the spinal rats were similar to the maximum values measured during hindlimb stepping in the intact rat, implying that the levels of extracellular glutamate in the dorsal horn may be downregulated by the supraspinal pathways.

Glutamate and activation of the ionotropic glutamatergic receptors induce locomotion in a variety of species. Application of NMDA and an EAA uptake blocker, dihydrokainic acid, produce a well coordinated locomotor pattern and administration of NMDA or non-NMDA antagonists (APV and CNQX) block fictive locomotion in the decerebrate cat (16). Further interesting experiments have shown that NMDA antagonists, applied during development, spare hindlimb function in older spinally transected rats (36). Although the potency of the excitatory glutamatergic system in generating alternating locomotor patterns is clear, the locus of this effect and the regional contribution of the different areas of the spinal cord remain to be determined.

A number of supraspinal pathways contain glutamate or are modulated by glutamatergic terminals found in the spinal cord. Descending glutamatergic pathways include the corticospinal, rubrospinal, pontospinal, and bulbospinal tracts (6,34,43,54), and projections from the rostral ventrolateral medulla, and the nucleus raphe magnus (55,62). Complete or partial transection of the spinal cord decreases total glutamate content by 10–28% in specific regions of the spinal cord (49,51,56). However, these studies do not distinguish between glutamate of neuronal or metabolic function and whether glutamate was present in the intra- or extracellular compartments of the spinal cord. The two-fold elevation in extracellular glutamate in the dorsal horn of the chronic spinally transected adult rat may reflect the lack of inhibition by descending pathways on the release of glutamate (53,65) or an increased gliosis following transection (30).

In conclusion, extracellular glutamate in the dorsal horn increased during hindlimb stepping in the freely moving intact rat. Extracellular glutamate also increased in the spinal rat during stepping and correlated with the number of steps taken by the spinal group. This suggests that extracellular glutamate in the dorsal horn is modulated at least in part by primary afferent depolarization during hindlimb stepping. Further studies need to be conducted to understand how the organization of the spinal cord following transection changes the biochemical milieu of the neural networks that generate stepping.

ACKNOWLEDGEMENTS

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