21. *The Effects of Dorsal Root Stimulation on the Release of Endogenous Glutamate from the Frog Spinal Cord*

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Glutamic acid and substance P are leading candidates among putative transmitters that have been proposed for the primary afferent pathways in the spinal cord.^{1),2)} However, no firm conclusion has been reached for the transmitter role of these substances. Substance P is several times more effective than L-glutamate in depolarizing motoneurons, but its action is slow compared to the natural transmitter.³⁾ Although the action of glutamate is rapid, the release of this substance has not been observed in the spinal cord following dorsal root stimulation.⁴⁾ Recent studies in the crayfish neuromuscular junction have provided strong evidence that glutamate is released and acts as the excitatory transmitter.^{5),6)} In the present experiments, therefore, the possibility as to whether the endogenous glutamate was released from the spinal cord following dorsal stimulation was explored.

Methods. The spinal cord and attached roots were carefully removed from bullfrogs (*Rana catesbeiana*) in ice-cold Ringer's solution bubbled with O₂ gas (with 5% CO₂). Experiments were undertaken on two types of preparations.

a) The hemisected cord. The preparation was placed in 200 μ l of the solution, which was collected every 10 min for the amino analysis.

b) The intra-arterially perfused spinal cord.⁷⁾ The preparation was placed in a bath and a glass cannula was inserted into the anterior spinal artery, and oxygenated Ringer solution was perfused at a rate of 300 μ l/10 min. The outflow from the vein was stored in the bath and collected every 10 min.

The bath solution was bubbled with O₂ gas and cooled to 7–9°C to prevent the uptake of amino acids. The Ringer's solution used was (mM): NaCl 115; KCl 2.0; CaCl₂ 2.0; pH was adjusted to 7.2 by HEPES.

The 8th ventral root was led into a separate compartment through a hole made on rubber film and the ventral root potential

(VRP) was recorded with a pair of calomel-wick electrodes. Dorsal roots (7–9th) were introduced into another compartment and placed on a pair of silver electrodes for nerve stimulation. To ensure a leak-proof separation of the compartment, all holes through which roots were led were coated with petroleum jelly. A train of 10 or 20 stimuli was applied every sec and the strength of the stimuli was adjusted to juxtamaximal for fast VRP.

The concentrations of glutamate and aspartate in the solution were measured by mass fragmentography using a gas chromatograph-mass spectrometer (GC-MS) (Finnigan Type 4000).⁶⁾ A negative ion detector was used for the mass fragmentography (negative ion-chemical ionization).⁸⁾ With this method glutamate and aspartate of less than 1 pmole in sample were measured.

Results. The results shown in Fig. 1 were obtained from an intra-arterially perfused spinal cord at 8°C. The filled columns represent glutamate (Glu) and aspartate (Asp) contents during 10 min resting period. The resting release of glutamate was relatively large, being 100–200 pmole per sample. A train of 10 stimuli was applied every sec to dorsal roots for 9 min and it produced VRP of about 200 μ V, as shown at the top of Fig. 1A. The amino acid content during nerve stimulation is indicated by the hatched columns. The glutamate efflux during dorsal root stimulation was increased by

Fig. 1. The effects of dorsal root stimulation on glutamate and aspartate release from intra-arterially perfused spinal cords. Each column represents the release during a collection period of 10 min. The solid columns are resting release and the hatched columns are those during dorsal root stimulation. Sample records of VRP are shown at the top. A and B were obtained from two different spinal cords.

about 30 pmole in excess of the background. Fig. 1B shows another example. Dorsal root stimulation at 10/sec increased glutamate efflux by about 80 pmole, whereas the second series of nerve stimuli at 20/sec was not very effective, with the increase in glutamate efflux being about 30 pmole. The background efflux of glutamate varied from preparation to preparation (118.67 ± 6.47 pmole (mean $\pm$ S.E.) ($n=46$)) and the increase in the glutamate efflux by dorsal root stimulation was small. However, when the glutamate efflux during nerve stimulation was compared with the mean value of the controls before and after nerve stimulation, a significant increase in the glutamate efflux was found in excess of the mean control release (15.78 ± 6.79 , $n=13$) ($P < 0.05$, paired t test).

The resting release of aspartate (Asp) was several times smaller than that of glutamate as shown in Fig. 1, the average value being 21.10 ± 1.01 pmole ($n=46$). The dorsal root stimulation produced a small but significant increase in aspartate, the net increase being 3.61 ± 1.93 pmole ($n=13$) ($P < 0.1$).

In the intra-arterially perfused preparations, the diffusion of amino acids into perfusate may be restricted by the blood-brain barriers. Therefore, a more conventional hemisected preparation was also used (Fig. 2). The resting release of glutamate was usually smaller than that observed in the intra-arterially perfused preparations. This may be partly attributed to the small volume of the hemisected spinal cords compared to the intra-arterially perfused preparations. The background release of glutamate averaged $31.21 \pm$

Fig. 2. The effects of dorsal root stimulation on glutamate and aspartate release from hemisected spinal cords. A and B were obtained from two different preparations.

3.42 pmole (n=19). The net increase of glutamate efflux brought about by dorsal root stimulation at 20/sec was 16.35 ± 6.62 pmole (n=5, $P < 0.1$). In this preparation the dorsal root stimulation produced an increase in the aspartate efflux on some occasions, but its value was variable and statistically not significant.

The effects of Ca on the glutamate and aspartate release were tested using the hemisected spinal cords. The Ca concentration of Ringer's solution was decreased to 0.1 mM by replacing Ca with Mg. When the spinal cord was soaked in the low Ca, high Mg solution for 30 min, the amplitude of VRP was decreased in most cases to less than 50 μ V. The net release of glutamate produced by dorsal root stimulation at 10/sec was significantly smaller in low Ca solution than that in the control (Table I). The net release of aspartate also showed a tendency to decrease in the low Ca solution, but this was statistically not significant.

Table I. The effects of low Ca on net release of glutamate and aspartate (10/sec stimulation) (mean $\pm$ S.E. pmole)

	Control	low Ca
Glutamate	13.44 ± 4.89 (n=11)	$1.64 \pm 3.14^*$ (n=7)
Aspartate	11.24 ± 6.31 (n=11)	2.34 ± 1.20 (n=7)

* $P < 0.1$ compared with control.

Discussion. The present experiments constitute the first demonstration that the dorsal root stimulation produced a calcium dependent release of endogenous glutamate from the spinal cord. Since the stimulus strength was adjusted to juxtamaximal in producing VRP, the primary afferents of larger sizes may be the major source of glutamate release. The contribution of aspartate was relatively small, but it might also have been acting as the transmitter in some parts of the spinal cord, e.g. the interneurons, because a significant release was found in the intra-arterially perfused preparations.

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