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RELEASE OF [MET^5]-ENKEPHALIN-ARG^6-GLY^7-LEU^8 IMMUNOREACTIVITY INTO RAT CEREBROSPINAL FLUID BY ELECTROCONVULSIVE SHOCK

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An association between electroconvulsive shock (ECS) and the endogenous opioid system has been suggested by several types of experiments. Repeated ECS activates rat proenkephalin biosynthesis in a number of discrete brain areas as assessed by increases in enkephalin peptides and in the mRNA coding for the proenkephalin precursor, particularly in the hypothalamus [1]. In addition, Tortella and Long [2] have shown that after ECS a substance which possesses anticonvulsant activity is released into the cerebrospinal fluid (CSF). The opioid nature of this substance was inferred by the ability of naloxone to reverse the anticonvulsant activity.

The purpose of the present experiment was to determine whether a proenkephalin-derived peptide, [met^5]-enkephalin-arg^6-gly^7-leu^8 (MERGL), could be released by ECS. Since chronic ECS causes an increase in tissue levels of enkephalin peptides [1], we administered ECS once per day for 15 days prior to removal of CSF. The stimulus parameters were 150 mA, 60 Hz for 200 msec, delivered via ear clip electrodes with a Wahlquist electroshock apparatus (Wahlquist Electronics, Salt Lake City, UT). The antibody used was directed against the C-terminus of MERGL and shows no cross-reaction against many other proenkephalin and prodynorphin-derived peptides [3]. Further details of the RIA procedure can be found in the table legend and in [3]. We determined whether the immunoreactivity in CSF could be accurately quantitated by assaying various volumes (0.5-50 μl) of boiled CSF after drying in a vacuum centrifuge. Figure 1 shows that the immunoreactivity in CSF caused a displacement of ^{125}I-MERGL tracer from the antibody which was parallel to that of authentic MERGL standards.

To test (1) whether there was an increase immediately after the ECS, and (2) whether this increase was sustained, we withdrew CSF from two experimental groups of rats: one at 30 min after the last shock (30-min group) and the second at 24 hr after the last shock (24-hr group); controls were not shocked. Table 1 shows that rat CSF contained a control level of immunoreactivity on the order of 1.5 pmoles/ml. After ECS a significant increase (67% over control, $P < 0.001$) in immunoreactivity occurred only in the 30-min group. In contrast, no significant increase occurred in the 24-hr group. An acute ECS (i.e. one shock only and withdrawal of CSF 30 min later) did not change the CSF content of MERGL immunoreactivity (mean pmoles/ml ± SEM; N = 6 per group; control: 1.43 ± 0.04 vs ECS: 1.51 ± 0.11). Thus, observation of the increase requires chronic application of the ECS as is required for the increase in tissue levels and for the therapeutic antidepressant effect.

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Figure 1. Parallel displacement of ^{125}I-MERGL tracer by immunoreactivity in CSF and MERGL standards.

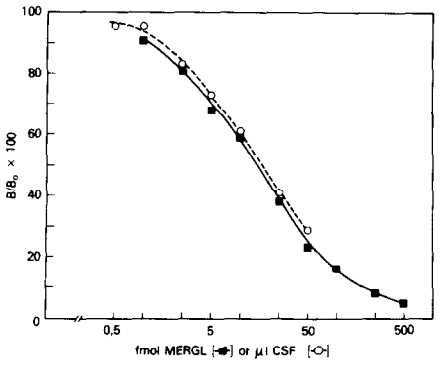

Table 1. Quantitative analysis of MERGL immunoreactivity (IR) in rat cisternal CSF after ECS.

	MERGL-like IR pmol/ml CSF	
CONTROL	1.45 ± 0.01	(9)
ECS 30 min	2.42 ± 0.17 *	(7)
ECS 24 hr	1.68 ± 0.10	(9)

*P < 0.001, ANOVA

CSF was withdrawn from the cisterna magna with a 27 gauge needle. The dura was exposed by a brief operative procedure under pentobarbital anesthesia; the rat heads were stabilized during the procedure by fixing them in a stereotaxic device. After boiling the CSF for 10 min, centrifugation at 12,000 x g for 10 min, aliquots were dried in a vacuum centrifuge and assayed for MERGL immunoreactivity as described previously [3]. In the displacement curve, various volumes of boiled CSF (0.5-50 µl) were dried and assayed in triplicate. The parallel displacement suggests that the CSF immunoreactivity is recognized in a similar fashion as the standards. The values in the table are the mean ± SEM of the MERGL content in CSF from rats that had been given ECS 30 min or 24 hr prior to withdrawal of CSF; the number of rats in each group is given in parentheses. Analysis of variance followed by the Scheffe test indicated that a significant ($P < 0.001$) increase occurred only in the 30-min group.

Further studies characterizing the CSF immunoreactivity with chromatographic methods are needed since Jackson et al. [4] have identified a high molecular weight MERGL peptide in rat CSF.

The present data demonstrate that ECS releases MERGL immunoreactivity from neuronal stores into the CSF and suggest that the increase reflects an acute, massive depolarization of enkephalin neurons induced by the ECS. This demonstration confirms that ECS not only acts upon enkephalin neurons to stimulate proenkephalin biosynthesis [1] but also results in a release of proenkephalin-derived opioid peptides. Such a release constitutes further evidence for a possible involvement of proenkephalin neurons in the therapeutic effect of ECS. In addition, it may be that the opioid substances with anticonvulsant activity described by Tortella and Long [2] are composed, in part, of MERGL-containing peptides.

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