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Effects of L-glutamate on cyclic AMP levels in slices from different areas of rat cerebral cortex with a chronic iron-induced focus.

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Abstract

The effect of L-glutamate of cyclic AMP levels in different areas of rat cerebral cortex was examined during the development of an iron-induced focus in the left sensorimotor cortex. The addition of L-glutamate resulted in increased cyclic AMP levels in slices from the quarter of cortex with the iron-induced focus. The levels lowered in slices from the quarter of cortex most remote from the focus. Thus L-glutamate showed stimulatory and inhibitory effects on the accumulation of cyclic AMP.

KEYWORDS: rat cerebral cortex, cyclic AMP, L-glutamate, iron-induced focus.

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— BRIEF NOTE —

**EFFECTS OF L-GLUTAMATE ON CYCLIC AMP LEVELS IN
SLICES FROM DIFFERENT AREAS OF RAT CEREBRAL
CORTEX WITH A CHRONIC IRON-INDUCED FOCUS**

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Abstract. The effect of L-glutamate on cyclic AMP levels in different areas of rat cerebral cortex was examined during the development of an iron-induced focus in the left sensorimotor cortex. The addition of L-glutamate resulted in increased cyclic AMP levels in slices from the quarter of cortex with the iron-induced focus. The levels lowered in slices from the quarter of cortex most remote from the focus. Thus L-glutamate showed stimulatory and inhibitory effects on the accumulation of cyclic AMP.

Key words : rat cerebral cortex, cyclic AMP, L-glutamate, iron-induced focus.

L-Glutamate elevates cyclic AMP level in incubated slices of guinea pig cerebral cortex (1,2). We designed this experiment to clarify the biochemical characteristics of cerebral cortex with a chronic iron-induced focus. The effect of L-glutamate on cyclic AMP levels in slices from different area of such cortex was studied.

Materials and methods. A chronic focus without generalized convulsions was induced by a single intracortical microinjection of a 5 μ l of 0.1 M FeCl₃ solution into the left sensorimotor cortex of male Wistar rat employing the method of Willmore *et al.* (3). The animals were sacrificed after confirmation of the appearance of epileptiform discharges in an electrocorticogram at early (about 1 week) and late (1 to 2 months) stages after the injection. The cerebral cortex was dissected into 4 parts, namely, left anterior, left posterior, right anterior and right posterior quarters. Each quarter of cortex was sliced in ice-cold Krebs-Henseleit Ringer bicarbonate buffer saturated with a mixture of 95% O₂ and 5% CO₂. The slices were preincubated at 37°C in 5 ml of the buffer for 20 min. At the end of preincubation, the suspension medium was replaced with 5 ml of fresh buffer solution containing 10 mM L-glutamate. After 10 min-incubation at 37°C, the suspension medium containing the test substance was replaced with 2.2 ml of

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cold 7% trichloroacetic acid and the mixture was homogenized in an ice-bath. Cyclic [^3H] AMP (0.05 pmole, about 2000 dpm for a recovery check) and 0.2 ml of 1 N HCl were added to 2 ml of the homogenate and centrifuged with $2000 \times g$ at 4°C for 15 min. The supernatant fraction was shaken with water-saturated ether, then the ether layer was removed. This treatment was repeated four times. Cyclic AMP in the aqueous fraction was isolated by Dowex 50W-X8 column chromatography (4), and assayed by a protein binding method (5). The protein content of the homogenate was estimated by the method of Lowry *et al.* (6).

Results. The addition of L-glutamate resulted in increase of cyclic AMP levels in the incubated slices from the left anterior quarter of cortex which contained the chronic focus due to microinjection of FeCl_3 solution. The increase of cyclic AMP level in both early and late stages was almost the same degree as those in a saline-injected group. Paradoxically, in the late stages, L-glutamate led to decreased cyclic AMP levels in the incubated slices from the right posterior quarter of cortex which was supposedly the most remote cortical area from the chronic focus in the left sensorimotor cortex (Table 1). Only the stimulatory effect of L-glutamate was observed in incubated slices from each quarter of rat cerebral cortex of the saline-injected group; 30 to 40 days after injection of saline, the level of cyclic AMP after addition of L-glutamate was about 150% that of the control.

TABLE 1. EFFECT OF L-GLUTAMATE ON CYCLIC AMP LEVELS IN INCUBATED SLICES FROM DIFFERENT QUARTERS OF CORTEX WITH A CHRONIC IRON-INDUCED FOCUS.

Stage	Cyclic AMP level (% of control)			
	Left cortex		Right cortex	
	Anterior	Posterior	Anterior	Posterior
Early	161 $\pm$ 22	108 $\pm$ 8	116 $\pm$ 10	99 $\pm$ 12
Late	148 $\pm$ 15	99 $\pm$ 28	87 $\pm$ 16	47 $\pm$ 8

Cyclic AMP level of control (100%) of incubated slices without L-glutamate from each quarter of cortex was about 50 pmoles per mg protein. Each value represents the mean $\pm$ S.E.M. of three different experiments.

Discussion. The mechanism of the dual action of L-glutamate on the cyclic AMP levels in slices from different areas of the rat cerebral cortex with a chronic iron-induced focus in the left sensorimotor area was not clarified. Apparently two types of L-glutamate receptors or sites exist in the cell membrane of the rat cerebral cortex, namely, one mediating the stimulation (G2) and the other mediating inhibition (G1) of cyclic AMP accumulation.

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