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## Regional difference in cyclic AMP response to adenosine of rat cerebral cortex with an iron-induced epileptic focus.

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# Regional difference in cyclic AMP response to adenosine of rat cerebral cortex with an iron-induced epileptic focus.\*

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## Abstract

A chronic epileptic focus not resulting in generalized convulsions was induced by a microinjection of FeCl<sub>3</sub> solution into the left anterior cortex of rats. Cyclic AMP accumulation in response to adenosine was examined in incubated slices from the cerebral cortex of animals that showed bilateral spike and slow wave complex in an electrocorticogram (ECoG) 30 to 60 days after the microinjection. Cyclic AMP accumulation after incubation with adenosine was most marked in slices from the left anterior quadrant of the cortex including a FeCl<sub>3</sub>-injected site. A medium response was observed in both the left posterior and the right anterior quadrants, but little or no response in the right posterior quadrant of the cortex.

**KEYWORDS:** rat cerebral cortex, iron-induced epileptic focus, cyclic AMP, adenosine

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— BRIEF NOTE —

**REGIONAL DIFFERENCE IN CYCLIC AMP RESPONSE TO  
ADENOSINE OF RAT CEREBRAL CORTEX WITH AN  
IRON-INDUCED EPILEPTIC FOCUS**

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*Abstract.* A chronic epileptic focus not resulting in generalized convulsions was induced by a microinjection of  $\text{FeCl}_3$  solution into the left anterior cortex of rats. Cyclic AMP accumulation in response to adenosine was examined in incubated slices from the cerebral cortex of animals that showed bilateral spike and slow wave complex in an electrocorticogram (ECoG) 30 to 60 days after the microinjection. Cyclic AMP accumulation after incubation with adenosine was most marked in slices from the left anterior quadrant of the cortex including a  $\text{FeCl}_3$ -injected site. A medium response was observed in both the left posterior and the right anterior quadrants, but little or no response in the right posterior quadrant of the cortex.

*Key words :* rat cerebral cortex, iron-induced epileptic focus, cyclic AMP, adenosine.

Chronic epileptic discharges are found in the ECoG of rats and cats which have been treated with an intracortical injection of iron compounds (1, 2). Previous results showed that when slices from rat cerebral cortex with an iron-induced epileptic focus were incubated with glutamate, the levels of cyclic AMP in different cortical areas were not uniform (3). In the present study, we compared the effect of adenosine on the cyclic AMP content of slices from four cortical areas of rats with a chronic iron-induced epileptic focus, since adenosine, like glutamate, has been shown to increase the level of cyclic AMP in cerebral cortical slices of rats (4, 5).

*Materials and Methods.* Experimental procedures were essentially the same as described previously (3), except that 0.5 mM adenosine was the test substance added to the incubation medium instead of glutamate. A chronic epileptic focus was induced by microinjection of 5  $\mu\text{l}$  of 0.1 M  $\text{FeCl}_3$  solution into the left sensorimotor cortex of male Wistar rats employing the method of

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Willmore *et al.* (1, 2). Animals were sacrificed after confirming the appearance of epileptic discharges in ECoG. The cerebral cortex was dissected into four parts: left anterior, left posterior, right anterior, and right posterior quadrants. After incubation of slices, cyclic AMP was isolated by Dowex 50W-X8 column chromatography (6) and assayed by a protein binding method (7). The protein content of the homogenate was estimated by the method of Lowry *et al.* (8).

**Results.** In most animals, spontaneous focal spike discharges were recorded at the frontal ECoG lead 1 week after the microinjection. In some cases, epileptic activities developed into bilateral spike and slow wave complex about 3 weeks after the microinjection, and the latter pattern often lasted for more than 10 weeks after the microinjection (Fig. 1). Behavioral features of epileptic activ-

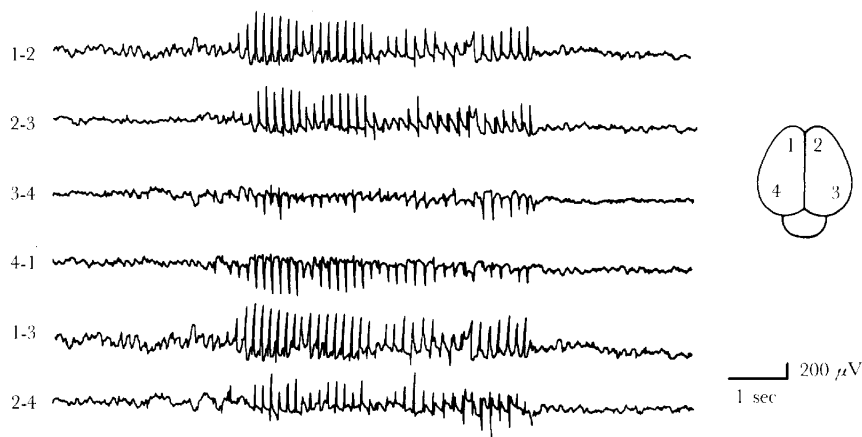


Fig. 1. ECoG of a rat with an iron-induced focus recorded 47 days after microinjection of  $\text{FeCl}_3$  solution.  $\text{FeCl}_3$  solution was injected into the left cortex at a point 1.5 mm rostral and 3.5 mm lateral to the bregma. Two of 4 stainless steel electrodes were located 3 mm rostral and 3 mm lateral to the bregma. The other two, 4 mm caudal and 4 mm lateral to the bregma.

TABLE 1. EFFECT OF ADENOSINE ON CYCLIC AMP LEVELS OF SLICES FROM FOUR QUADRANTS OF CEREBRAL CORTEX.

| Group                             | Cyclic AMP (% of control) |              |              |                  |
|-----------------------------------|---------------------------|--------------|--------------|------------------|
|                                   | Left cortex               |              | Right cortex |                  |
|                                   | Anterior                  | Posterior    | Anterior     | Posterior        |
| $\text{FeCl}_3$ solution-injected | $261 \pm 16^*$            | $206 \pm 12$ | $160 \pm 14$ | $104 \pm 4^{**}$ |
| Saline-injected                   | $207 \pm 9$               | $216 \pm 26$ | $208 \pm 22$ | $203 \pm 27$     |

Animals were sacrificed 30 to 60 days after microinjection of  $\text{FeCl}_3$  solution or saline. The cyclic AMP content of control slices incubated without adenosine was about 30 pmoles per mg protein irrespective of the cortical areas. Values represent the mean  $\pm$  S.E.M. of five different experiments.  $^*P < 0.02$ ,  $^{**}P < 0.01$  compared with saline-injected animals (Student's *t*-test).

ities were restricted to intermittent twitching of face or neck muscles. In animals which showed the bilateral spike and slow wave complex 30 to 60 days after the microinjection, addition of adenosine to the incubation medium caused 2- to 3-fold increase in cyclic AMP levels of slices prepared from the left anterior quadrant of the cortex containing the  $\text{FeCl}_3$ -injected site (Table 1). Adenosine was less effective in the left posterior and right anterior quadrants, and nearly ineffective in the right posterior quadrant. Table 1 also shows that regional difference in the adenosine effect was not observed in the cortical slices from saline-injected animals.

*Discussion.* It has been suggested that there is some relationship between epileptic activity and the level of cyclic nucleotides in several regions of the brain (9). Our previous results suggest that regional change in the cyclic AMP-generating system of the cerebral cortex was involved in the process of a cortical ferric focus with minimal epileptic activities (3). A similar effect was shown in the present study.

Recently, two types of adenosine receptors were reported in cultured cells from mouse brain: an A1 receptor mediating inhibition and an A2 receptor mediating stimulation of cyclic AMP accumulation (10, 11). An adenosine receptor mediating inhibition of adenylate cyclase was found in rat cerebral cortex (12). Furthermore, it was reported that pentylenetetrazol seizure resulted in a decrease in the adenosine binding sites of the cerebellum (13). In relation to these reports, the present results lead us to the assumption that modification of cell membrane functions in the cerebral cortex including some change in adenosine receptors is involved in the development of an iron-induced epileptic focus.

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