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Elicitation of cyclic AMP accumulation by norepinephrine in the iron-induced epileptogenic cerebral cortex of rats.

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Abstract

A microinjection of ferric chloride solution into the left frontal cortex of rats induced epileptic discharges which were recorded by electrocorticography. In animals having such electrographic seizure activity 30 to 60 days after the injection, the accumulation of cyclic AMP elicited by norepinephrine was examined in slices from four cortical regions. The accumulation was significantly greater in the left anterior area, into which region the ferric chloride solution was injected, than in the right anterior area. There was also a tendency for greater norepinephrine-elicited accumulation of cyclic AMP to occur in the left posterior area than in the right posterior area.

KEYWORDS: rat cerebral cortex, iron-induced epileptic discharge, cortical slices, cyclic AMP, norepinephrine

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— BRIEF NOTE —

ELICITATION OF CYCLIC AMP ACCUMULATION BY NOREPINEPHRINE IN THE IRON-INDUCED EPILEPTOGENIC CEREBRAL CORTEX OF RATS

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Abstract. A microinjection of ferric chloride solution into the left frontal cortex of rats induced epileptic discharges which were recorded by electrocorticography. In animals having such electrographic seizure activity 30 to 60 days after the injection, the accumulation of cyclic AMP elicited by norepinephrine was examined in slices from four cortical regions. The accumulation was significantly greater in the left anterior area, into which region the ferric chloride solution was injected, than in the right anterior area. There was also a tendency for greater norepinephrine-elicited accumulation of cyclic AMP to occur in the left posterior area than in the right posterior area.

Key words : rat cerebral cortex, iron-induced epileptic discharge, cortical slices, cyclic AMP, norepinephrine.

The cyclic nucleotide level in the brain has been reported to be elevated during seizure activity, such as during electroconvulsion (1, 2) or pentylenetetrazol seizure (3). An elevation in the cyclic nucleotide level was also found in acute focal epilepsy (4, 5). In addition to these *in vivo* changes in the contents of cyclic AMP and cyclic GMP in epileptic cortex, an *in vitro* change in the contents of cyclic AMP was found to occur in response to putative neurotransmitters or neuromodulators in slices from rat cerebral cortex with a local epileptic focus. Using cortical slices from rats with a chronic epileptic focus, our previous study showed that there was a regional difference in the cyclic AMP response to adenosine (6). Besides adenosine, the accumulation of cyclic AMP has been reported to be elicited by addition of norepinephrine to cortical tissues isolated from rats (7). In the present study, the cyclic AMP accumulation elicited by norepinephrine was compared in slices from different cortical regions of rats with an iron-induced epileptic focus.

Materials and Methods. Experimental procedures were essentially the same as described previously (6, 8). Male Wistar rats weighing 220-270 g received a microinjection of 5 μl of 0.1 M $FeCl_3$ solution into the left frontal cortex (Fig. 1) employing the methods of Willmore *et al.* (9). Stainless steel electrodes were im-

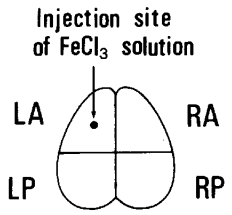

Fig. 1. Schematic diagram of injection site and cortical regions. LA, RA, LP and RP indicate the left anterior, right anterior, left posterior, and right posterior cortical quadrants, respectively. The injection site of the $FeCl_3$ solution was within LA.

planted in the cranial bone for electrocorticogram (ECoG) recording. After confirming the appearance of epileptic discharges in ECoGs, animals were sacrificed by decapitation. The cerebral cortex was dissected into four quadrants (Fig. 1) and chopped with a McIlwain tissue chopper. After a 30-min preincubation, the chopped slices from each quadrant were incubated for 10 min at 37 ℃ in 5 ml of Krebs-Ringer bicarbonate buffer with or without 0.1 mM norepinephrine. The suspensions were constantly aerated with 95 % O_2-5 % CO_2 throughout both the preincubation and incubation. Following the incubation, cyclic AMP was purified by column chromatography (10) and assayed by a protein binding method (11). The protein content was determined by the method of Lowry *et al.* (12).

Results and Discussion. An intracortical injection of ferric chloride solution produced spike and slow wave complexes in ECoGs as reported previously (6). The electrographic seizure activity lasted for more than two months in a high percentage of the cases. Behavioral manifestation of the seizure activity was only the intermittent twitching of the face or neck muscles. In animals having such spike and slow wave complexes 30 to 60 days after the injection, cyclic AMP levels of cortical slices were elevated by addition of 0.1 mM norepinephrine 4- to 6-fold, which is in agreement with the results of other workers (7). The elevated levels of cyclic AMP elicited by norepinephrine, however, were not the same in the four cortical quadrants (Fig. 2). In the two anterior quadrants of the cortex, the norepinephrine-elicited accumulation of cyclic AMP was significantly greater on left side, which included the injection site, than on right side. In the two posterior quadrants of the cortex, the norepinephrine-elicited accumulation of cyclic AMP was also somewhat greater on the left side than on the right. The cortex of saline-injected animals, from which normal ECoGs were recorded, did not show such regional differences in accumulation of cyclic AMP elicited by norepinephrine (Fig. 2).

It has been demonstrated that treatment of rats with 6-hydroxydopamine (13, 14) or reserpine (15, 16) results in a higher response of cyclic AMP accumulation to norepinephrine in cerebral cortical slices. Therefore, there may be compensatory enhancement in the response of the norepinephrine-sensitive cyclic AMP-generating system. In the epileptic cortex of rats in which focal seizures were induced by freezing, Walker *et al.* (4) found that an increase in adenylate cyclase activity and a decrease in phosphodiesterase activity occurred and that

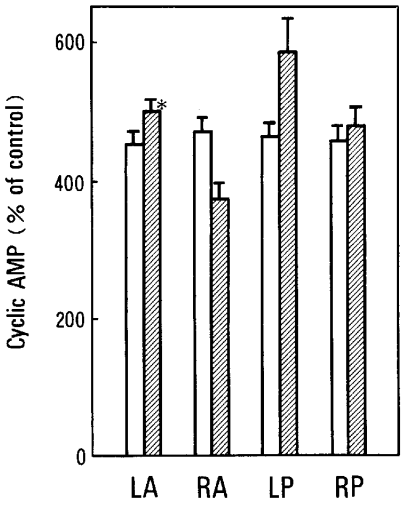

Fig. 2. Norepinephrine-elicited accumulation of cyclic AMP in slices from four regions of rat cerebral cortex. Animals were sacrificed 30 to 60 days after injection of $FeCl_3$ solution (▨) or saline (□). The cyclic AMP content of slices after incubation without norepinephrine was about 20 pmol/mg protein. Values are expressed as the mean ± S.E.M. of five to eight different experiments. *Significantly different from the value of the contralateral area in rats injected with $FeIC_3$ solution, $p<0.01$ by Student's *t*-test. LA, RA, LP and RP indicate the cortical quadrants as in Fig. 1.

cyclic AMP levels were elevated in the cortex, especially in the region of epileptogenic lesions. The results presented here demonstrate that there is a regional difference in the cyclic AMP response to norepinephrine in slices of rat cerebral cortex with iron-induced electrographic seizure activity. These results suggest that the properties of cell membrane components, including the norepinephrine receptor coupled to the adenylate cyclase system in the cortex, may be modified in the process leading to chronic epileptic foci.

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