Acta Medica Okayama

Volume 39, *Issue* 6 1985 *Article* 9

DECEMBER 1985

Regional difference in depolarization-elicited accumulation of cyclic AMP in cobalt-induced epileptic cortex of the rat.

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Abstract

The appearance of epileptiform discharges in electrocorticograms was induced by a unilateral injection of CoCl2 solution into the sensorimotor cortex of rats. Accumulation of cyclic AMP elicited by ouabain or a high concentration of potassium ions was determined in slices from different cortical areas of rats 9 or 10 days after the injection. In the anterior cortex, the depolarization-elicited accumulation of cyclic AMP was significantly higher in the cortical area ipsilateral to the injection site than in the contralateral cortical area. In the posterior cortex, a similar but not significant difference in the accumulation of cyclic AMP was noted.

KEYWORDS: cobalt-induced epilepsy, rat cerebral cortex, slices, depolarization, cyclic AMP

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*PMID: 3004112 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 39, (6), 489-492 (1985)

—— BRIEF NOTE ——

REGIONAL DIFFERENCE IN DEPOLARIZATION-ELICITED ACCUMULATION OF CYCLIC AMP IN COBALT-INDUCED EPILEPTIC CORTEX OF THE RAT

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Received August 30, 1985

Abstract. The appearance of epileptiform discharges in electrocorticograms was induced by a unilateral injection of $CoCl_2$ solution into the sensorimotor cortex of rats. Accumulation of cyclic AMP elicited by ouabain or a high concentration of potassium ions was determined in slices from different cortical areas of rats 9 or 10 days after the injection. In the anterior cortex, the depolarization-elicited accumulation of cyclic AMP was significantly higher in the cortical area ipsilateral to the injection site than in the contralateral cortical area. In the posterior cortex, a similar but not significant difference in the accumulation of cyclic AMP was noted.

Key words : cobalt-induced epilepsy, rat cerebral cortex, slices, depolarization, cyclic AMP.

Elevated levels of cyclic AMP or cyclic GMP have been reported in the epileptic cortex such as in that with an ethyl chloride focus (1) or penicillin focus (2). These studies have led us to the speculation that the regulatory function of the cyclic AMP-generating system plays a role in abnormal neuronal activity. Recently, we have shown that, in the iron-induced epileptic cortex, there is a regional difference in the accumulation of cyclic AMP elicited by adenosine, norepinephrine, glutamate, and depolarizing agents (3-6).

A topical application of cobalt is widely used to induce recurrent epileptic activity. Several forms of cobalt for the induction of epileptic activity have been reported: powder, gelatine, and wire. In addition, it has been reported that iontophoretic application of a $CoCl_2$ solution induces epileptic activity (7). In this study, a modification of the iontophoretic method was adopted, and accumulation of cyclic AMP in response to ouabain or a high concentration of potassium ions was compared in slices from different areas of the cobalt-induced epileptic cortex of rats.

Materials and Methods. Male Wistar rats weighing 190-240 g were used. A trephine hole was made in the cranial bone over the left sensorimotor cortex at a point 1.5 mm rostral and 3.5 mm lateral to the bregma. The tip of a microsyringe

needle was inserted through the hole to a depth of 1.5 mm from the dorsal surface of the bone, and 5 μl of 100 mM $CoCl_2$ solution was injected. In control rats, an equal volume of saline was injected in the same way. Stainless steel electrodes for recording electrocorticograms (ECoGs) were implanted in the cranial bone, and ECoGs were recorded daily under unrestrained conditions.

Rats were sacrificed by decapitation 9 or 10 days after the injection. The cerebrum was rapidly extirpated, and the cortex was divided into four parts: the left anterior, right anterior, left posterior, and right posterior quadrants. The injection site was in the left anterior quadrant. Cross-chopped cortical slices were prepared from each quadrant. The incubation and assay procedures were essentially the same as described previously (5, 6). Briefly, cortical slices were preincubated for 30 min in Krebs-Ringer bicarbonate-glucose solution, and then the slices were incubated for 10 min in fresh solution alone or solution containing 0.1 mM ouabain or 100 mM KCl. The preincubation and incubation were performed at 37℃ with constant aeration with a mixture of 95 % O_2 and 5 % CO_2. After the incubated slices were homogenized, cyclic AMP was purified by column chromatography (8) and determined using an assay kit available from Amersham International, based on the protein binding method of Gilman (9). Protein was determined by the method of Lowry *et al.* (10) with bovine serum albumin as the standard.

Results and Discussion. An intracortical injection of $CoCl_2$ solution induced the appearance of ECoG spike discharges. The abnormal ECoGs were not observed in the control rats.

The cyclic AMP contents of incubated cortical slices from rats 9 or 10 days after injection of $CoCl_2$ solution or saline are summarized in Table 1. Cyclic AMP contents of slices after incubation without any additions were not significantly different among the four cortical areas. The cyclic AMP contents of slices were elevated 4- to 6-fold by incubation with 0.1 mM ouabain or 100 mM KCl. In rats receiving the injection of $CoCl_2$ solution, non-uniformity was detected in the elevated levels of cyclic AMP. The cyclic AMP contents after incubation with ouabain were significantly higher in the left anterior area than in the right anterior area. Similar results were obtained when the slices of the anterior cortex were incubated with a high concentration of KCl. In the posterior cortical areas, the cyclic AMP contents after incubation with ouabain or a high concentration of KCl were slightly higher on the left side than on the right, but the difference was not significant. In cortical slices of control rats, there was no regional difference in the accumulation of cyclic AMP.

Numerous studies have suggested a role of cyclic nucleotides in the physiological regulation of synaptic transmission in the central nervous system. In slice preparations, it has been reported that various compounds including depolarizing agents are effective in eliciting cyclic AMP accumulation (11). The results presented here show that, in cortical slices of rats receiving an injection of $CoCl_2$

TABLE 1. CYCLIC AMP CONTENTS OF INCUBATED SLICES FROM FOUR AREAS OF RAT CEREBRAL CORTEX

Treatment and cortical area	Cyclic AMP (pmol/mg protein)		
	No addition	Ouabain	KCl
$CoCl_2$ solution injection			
Left anterior	10.3 ± 1.2	66.1 ± 7.3[b]	56.7 ± 6.3[a]
Right anterior	9.6 ± 0.9	39.7 ± 4.1	37.4 ± 4.3
Left posterior	9.8 ± 1.4	57.6 ± 4.9	48.0 ± 5.2
Right posterior	10.1 ± 1.2	46.5 ± 4.4	40.5 ± 3.9
Saline injection			
Left anterior	10.8 ± 0.9	57.2 ± 6.1	54.8 ± 5.9
Right anterior	11.5 ± 1.1	58.7 ± 5.6	51.6 ± 4.8
Left posterior	10.7 ± 1.3	54.6 ± 6.4	48.9 ± 5.6
Right posterior	11.1 ± 1.0	59.8 ± 5.7	52.7 ± 4.7

Cortical slices were prepared from rats 9 or 10 days after injection of $CoCl_2$ solution or saline. Concentrations of ouabain and KCl were 0.1 and 100 mM, respectively. Values represent the mean ± SEM of 4-6 different experiments. [a] $p<0.05$, [b] $p<0.02$ compared with the value of the contralateral cortical area (Student's t test).

solution, ouabain or a high concentration of potassium ions elicited cyclic AMP accumulation in agreement with previous studies (6, 12, 13). It should be noted that the cyclic AMP accumulation differed regionally in the cobalt-induced epileptic cortex in this study. The significant difference was detected only in the anterior half of the cortex, in one side of which $CoCl_2$ solution was injected. These results suggest that the depolarization-elicited accumulation of cyclic AMP corresponds to the excitability level of the cortex, though little is known about the elicitation mechanism. Further, the regional difference may be associated with alterations in Na^+, K^+-ATPase activity, because a decrease in the enzyme activity has been reported in the cortex with a cobalt-induced epileptic focus (14).

The regional difference in the cyclic AMP accumulation appears to be due primarily to a decrease in the right anterior cortical area, as compared with the corresponding cortical area of control rats (Table 1). No functional implications of the apparent decrease are known at present. Further electrographic investigations may elucidate the nature of fluctuations in cyclic AMP accumulation in relation to the fundamental events in cobalt-induced epilepsy.

The results of this study suggest that changes in cell membrane components relating to cyclic AMP generation in response to depolarizing agents occur during the process of cobalt-induced epileptic focus, as in iron-induced epilepsy (6). Thus, it seems likely that some changes in the cyclic AMP-generating system are common to several experimental models of epilepsy.

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