Acta Medica Okayama

Volume 41, *Issue* 6 1987 *Article* 8

DECEMBER 1987

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Abstract

Cyclic AMP accumulation in response to norepinephrine was examined in slices of rat cerebral cortex the day after a unilateral application of anodal current of 0.3, 3.0 or 30 microA to the surface of the sensorimotor cortex. When 3.0 microA was applied, the norepinephrine-elicited accumulation of cyclic AMP was greater in the cortical area including the application point than in either the contralateral cortical area or non-polarized control cortical slices. The changes in cyclic AMP accumulation are discussed in relation to the role of the direct current in producing functional changes in the cortex.

KEYWORDS: rat cerebral cortex, norepinephrine, cyclic AMP, polarization

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*PMID: 2830766 [PubMed - indexed for MEDLINE]
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Acta Med Okayama 41 (6) 285-288 (1987)

— Brief Note —

Norepinephrine-Elicited Accumulation of Cyclic AMP in Slices of Rat Cerebral Cortex Polarized with a Weak Anodal Current

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Cyclic AMP accumulation in response to norepinephrine was examined in slices of rat cerebral cortex the day after a unilateral application of anodal current of 0.3, 3.0 or 30 μA to the surface of the sensorimotor cortex. When 3.0 μA was applied, the norepinephrine-elicited accumulation of cyclic AMP was greater in the cortical area including the application point than in either the contralateral cortical area or non-polarized control cortical slices. The changes in cyclic AMP accumulation are discussed in relation to the role of the direct current in producing functional changes in the cortex.

Key words : rat cerebral cortex, norepinephrine, cyclic AMP, polarization

Prolonged application of weak anodal current to the surface of the cortical motor area (anodal polarization) has been shown to cause a characteristic motor response. It has been suggested that this motor response is due to a dominant focus which may be induced at the polarized point (1). The effects of the anodal polarization have been reported to last for several weeks after the polarization (2). Primary alterations which develop into the dominant focus have been speculated to originate in the cortex at or around the polarized point and to last for a long time. However, little is known about the central mechanisms of the process leading to the dominant focus. We believe that one episode of polarization may leave some traces in the cortex, but that repetitions of the polarization are necessary to induce a stable behavioral change.

It has been shown that cyclic AMP plays an important role as a second messenger in the regulatory function of the nervous system, and that its accumulation in rat cerebral cortical slices is elicited by putative neurotransmitters or neuromodulators such as norepinephrine (3). The present investigation is concerned with the cyclic AMP accumulation in response to norepinephrine in slices from four cortical areas of rats after one polarization event.

Male Wistar rats weighing 180-220 g were used. Under ether anesthesia, two silver electrodes (1 mm in diameter) were implanted in the cranial bone such that their tips rested on the dura mater over the sensorimotor cortex, and a stainless steel electrode was implanted in the nasal bone. These electrodes were fixed tightly to the bone with a dental resin. More than one week after the surgery, the rats were polarized under unrestrained conditions : An anodal current of 0.3, 3.0 or 30 μA was continuously applied to the left sensorimotor

Produced by The Berkeley Electronic Press, 1987

cortex for 30 min with a cortical electrode using a nasal indifferent electrode.

On the day following the polarization, rats were killed by decapitation, and the cerebral cortex was extirpated and dissected into four parts : the left anterior, right anterior, left posterior, and right posterior cortical areas. The polarized point was in the left anterior cortical area. Non-polarized control rats were killed at comparable times after the surgery. Cross-chopped cortical slices of 400 μm thickness were prepared with a McIlwain tissue chopper. The slices from each cortical area were preincubated in 5 ml of Krebs-Ringer bicarbonate-glucose buffer for 60 min at 37℃. After the preincubation, the medium was replaced with 5 ml of fresh buffer, and the slices were incubated for 10 min at 37℃ with or without 0.1 mM norepinephrine. The slice suspensions were constantly gassed with 95% O_2-5% CO_2 throughout the preincubation and incubation. At the end of the incubation, the medium was removed, and 2.5 ml of ice-cold 7% trichloroacetic acid was added to the slices. After homogenization of the resultant mixture, cyclic AMP was purified by Dowex 50W-X8 column chromatography (4), and its concentration was determined with a cyclic AMP assay kit (Amersham International plc), which is based on protein binding (5). Protein content was determined by the method of Lowry *et al.* (6).

During and after the anodal polarization, the rats did not show any abnormal motion spontaneously or in response to extraneous

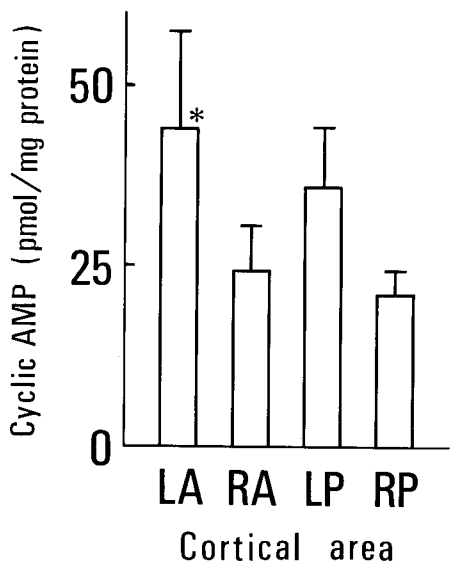

Fig. 1 Norepinephrine-elicited accumulation of cyclic AMP in the cerebral cortical slices of rats polarized with 3.0 μA. Cerebral cortical slices were incubated with 0.1 mM norepinephrine. LA, RA, LP, and RP indicate the left anterior, right anterior, left posterior and right posterior cortical areas, respectively. Values represent the mean ± SEM of 8 to 12 different experiments. *: Significantly greater than the value of the contralateral area determined by Student's t test. $p < 0.05$.

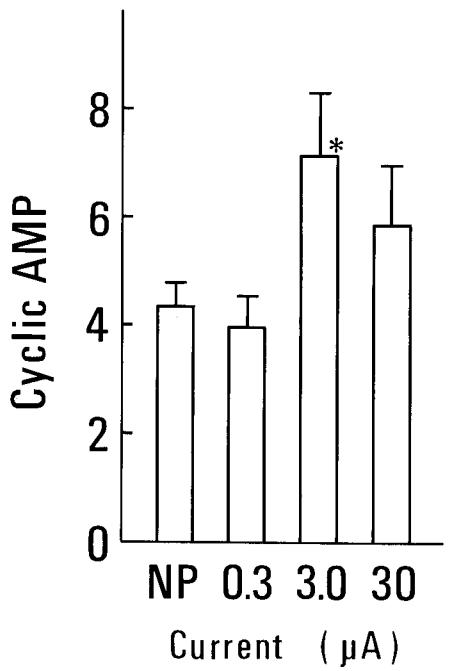

Fig. 2 Norepinephrine-elicited accumulation of cyclic AMP in slices of the left anterior cortical area including the polarized point. NP indicates non-polarization. Values represent the mean ± SEM of the ratios of cyclic AMP content in 0.1 mM norepinephrine-treated slices to that in untreated slices of 8 to 12 different experiments. *: Significantly different from the value of non-polarized control determined by Student's t test. $p < 0.05$.

stimuli. Fig. 1 shows norepinephrine-elicited accumulation of cyclic AMP in the cerebral cortical slices of rats polarized with 3.0 μA. The cyclic AMP content was significantly greater in the left anterior cortical area including the polarized point than in the right anterior area. In the posterior cortical areas, there was no significant difference in the cyclic AMP contents between the left and the right side. In rats polarized with 0.3 or 30 μA, the cyclic AMP contents did not significantly differ among the four cortical areas. There were no regional differences in cyclic AMP contents of cortical slices incubated without norepinephrine under any polarization condition. In non-polarized control rats, there was no regional difference in cyclic AMP contents after incubation with or without norepinephrine.

Fig. 2 shows the effects of currents of different intensities on the norepinephrine-elicited accumulation of cyclic AMP in slices of the left anterior cortical area including the polarized point. The cyclic AMP accumulation in slices of cortex polarized with 3.0 μA was significantly greater than that in the non-polarized control. The cyclic AMP accumulation in slices of cortex polarized with 30 μA was somewhat greater than that in the non-polarized control, but the difference was not significant. The cyclic AMP accumulation in slices of cortex polarized with 0.3 μA did not differ from that in the non-polarized control.

Regional differences in cyclic AMP accumulation were caused by polarization with 3.0 μA, but not by polarization with 0.3 or 30 μA, in agreement with the observations that current intensities of 1.0 to 10 μA were the most effective in inducing a behavioral change (7). Although the reason for our results is unknown, it may be that 0.3 μA was too weak to alter the cyclic AMP-generating system, while 30 μA altered the cyclic AMP-generating system in the cerebral cortex too much. Another possibility is that the alteration in the cyclic AMP-generating system caused by 30 μA differs from that caused by 3.0 μA.

Bindman *et al.* (8) reported that anodal polarizing currents of the order of 0.1-0.5 μA increased the firing of neurons and that the enhanced activity lasted for many hours. However, in the present study, a current of 0.3 μA did not alter cyclic AMP accumulation. The cause of this discrepancy is obscure, but it could result partially from the different experimental procedures. We have observed that regional differences in the cyclic AMP-generating system exist in the cerebral cortex of rabbits with dominant flexions of the forelimb (9). It appears reasonable to assume that the cyclic AMP-generating system of the rat cortex was altered prior to inducement of a behavioral change, since regional differences in cyclic AMP accumulation were observed in the rats polarized once with 3.0 μA and since the rats did not show any abnormal motion. Thus, it seems likely that local alteration of the cyclic AMP-generating system in the cerebral cortex is related to fundamental events in the genesis of a dominant focus.

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Received: July 7, 1987
Accepted: September 22, 1987

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