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# Repeated application of anodal direct current produces regional dominance in histamine-elicited cyclic AMP accumulation in rabbit cerebral cortex.

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## Abstract

A unilateral 30-min application of anodal direct current to the promotor cortex of rabbits was repeated 10 times, and cyclic AMP accumulation in response to histamine was investigated in slices of different cortical areas. Polarization with 1.0 microA decreased the cyclic AMP accumulation in the cortical area contralateral to the polarization, by which regional dominance in cyclic AMP accumulation was produced in the polarized cortex. In contrast, the regional difference in cyclic AMP accumulation was reversed when 10.0 or 30.0 microA was applied. The histamine-elicited accumulation of cyclic AMP was almost completely inhibited by the selective H2-receptor antagonist cimetidine. These results suggest that repeated anodal polarization regionally alters H2-receptor-mediated cyclic AMP generation in the cortex depending on the intensity of the polarizing currents and this pattern of cyclic AMP accumulation is responsible for the characteristic motor behavior induced by anodal polarization.

**KEYWORDS:** anodal polarization, cyclic AMP, histamine, cerebral cortex, rabbit

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— Brief Note —

# Repeated Application of Anodal Direct Current Produces Regional Dominance in Histamine-Elicited Cyclic AMP Accumulation in Rabbit Cerebral Cortex

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A unilateral 30-min application of anodal direct current to the premotor cortex of rabbits was repeated 10 times, and cyclic AMP accumulation in response to histamine was investigated in slices of different cortical areas. Polarization with 1.0 $\mu$A decreased the cyclic AMP accumulation in the cortical area contralateral to the polarization, by which regional dominance in cyclic AMP accumulation was produced in the polarized cortex. In contrast, the regional difference in cyclic AMP accumulation was reversed when 10.0 or 30.0 $\mu$A was applied. The histamine-elicited accumulation of cyclic AMP was almost completely inhibited by the selective $H_2$-receptor antagonist cimetidine. These results suggest that repeated anodal polarization regionally alters $H_2$-receptor-mediated cyclic AMP generation in the cortex depending on the intensity of the polarizing currents and this pattern of cyclic AMP accumulation is responsible for the characteristic motor behavior induced by anodal polarization.

**Key words:** anodal polarization, cyclic AMP, histamine, cerebral cortex, rabbit

Application of weak anodal direct current to the surface of the motor or premotor cortex is known to induce long-lasting changes in peripheral motor activities such as flexions and struggles of the forelimbs. This behavioral activity has been shown to persist from several hours to weeks after the polarization (1, 2). This phenomenon may be due to the formation of an excitation focus, which is called the dominant focus, in the cortex by anodal polarization (3). The unitary firing activity of cortical neurons was enhanced by anodal polarization, and the enhancement lasted from several minutes to hours after the polarization (4-6). The polarizing currents are suggested to have long-lasting effects on synaptic transmission following the increased neuronal activity in the cortex.

Putative neurotransmitters or neuromodulators are known to elicit cyclic AMP accumulation *in vitro* in the mammalian central nervous system (7). Histamine has been shown to be one of the most potent inducers of cyclic AMP accumulation in rabbit cerebral cortical slices (8-10). Previous findings have suggested that anodal polarization alters the activity of cyclic AMP-generating systems in the cerebral cortex (11, 12); however, little is known about the precise mechanisms in rabbits. In the present study, we investigated the cyclic AMP accumulation in response to histamine in cortical slices of rabbits receiving repeated anodal polarization to the unilateral premotor cortex. The effects of a histamine $H_2$-receptor antagonist on the cyclic AMP accumulation were also analyzed.

Male Japanese white rabbits weighing about 3.0 kg were used. The rabbits were maintained on a 12-h light-dark cycle at 20-24°C, with food and water *ad libitum*. The surgical procedures and polarization schedules were essentially the same as described previously (13). Briefly, under sodium pentobarbital anesthesia (40 mg/kg, i.p.), silver electrodes (1 mm in diameter) were bilaterally implanted into the cranial bone over the premotor cortex. Under unanesthetized and restrained conditions in a wooden pillory, anodal direct current of 0.3, 1.0, 10.0,

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**Table 1** Cyclic AMP contents in rabbit cortical slices incubated with or without histamine and cimetidine after polarization with different current intensities

| Current intensity and cortical area | Cyclic AMP (pmol/mg protein) | | |
|---|---|---|---|
| | No addition | 100 $\mu$M Histamine | 100 $\mu$M Histamine plus 100 $\mu$M Cimetidine |
| Non-polarization | | | |
| Left | 3.7 ± 0.5 | 71.6 ± 12.4 | 5.3 ± 0.3 |
| Right | 3.8 ± 0.2 | 73.3 ± 6.4 | 5.2 ± 0.4 |
| 0.3 $\mu$A | | | |
| Left | 3.4 ± 0.5 | 74.7 ± 17.7 | 6.3 ± 0.7 |
| Right | 3.9 ± 0.8 | 74.2 ± 18.3 | 5.8 ± 2.2 |
| 1.0 $\mu$A | | | |
| Left | 3.9 ± 0.4 | 63.6 ± 7.0[b] | 8.7 ± 1.1 |
| Right | 3.7 ± 0.4 | 33.1 ± 7.6[a] | 6.0 ± 1.5 |
| 10.0 $\mu$A | | | |
| Left | 3.2 ± 0.8 | 28.3 ± 1.1[a,b] | 3.4 ± 0.2 |
| Right | 3.4 ± 0.2 | 53.2 ± 8.7 | 4.1 ± 1.4 |
| 30.0 $\mu$A | | | |
| Left | 3.2 ± 0.1 | 32.3 ± 5.2[a,c] | 5.4 ± 0.2 |
| Right | 2.8 ± 0.4 | 67.0 ± 3.4 | 6.1 ± 0.4 |

Each value represents the mean ± SEM of 3 to 6 different rabbits.
Significantly different from non-polarized control at [a] $P < 0.05$.
Significantly different from contralateral cortical area at [b] $P < 0.05$ and [c] $P < 0.01$.

or 30.0 $\mu$A was applied for 30 min to the left premotor area using a silver plate on the left ear lobe as the cathode. The 30-min anodal polarization was repeated 10 times at intervals of 2 to 3 days at least 1 week after the surgery. For the control, rabbits were sham operated but no current was applied.

About 24 h after the last polarization trial, rabbits were decapitated using a special guillotine. The cerebrum was quickly extirpated, and the cortical areas under the two electrodes (about 0.8 $cm^2$) were dissected and sliced with a McIlwain tissue chopper to a thickness of 400 $\mu$m. The cortical slices (about 4 mg of protein) were preincubated for 60 min in 5 ml of a Krebs-Ringer bicarbonate-glucose solution. After the preincubation, the slices were further incubated for 20 min in 5 ml of fresh solution with or without 100 $\mu$M histamine, which was present for the final 15 min. When required, 100 $\mu$M cimetidine was present in the slice suspension throughout the 20-min incubation, because the same concentration of cimetidine was used to examine its effect on histamine-elicited accumulation of cyclic AMP (14). The preincubation and incubation were performed at 37 °C with constant aeration with 95 % $O_2$/5 % $CO_2$. The incubation was stopped by decanting the solution and adding 2.3 ml of ice-cold 7 % trichloroacetic acid to the slices. After homogenization, cyclic AMP was purified by Dowex 50W-X8 column chromatography and assayed using a cyclic AMP assay kit (Amersham International) based on the protein-binding method. The protein content was determined by the method of Lowry *et al.* (15) using bovine serum albumin as the standard. Cyclic AMP content in cortical slices was expressed as pmol/mg protein. Statistical significance was evaluated by one-way analysis of variance or Student's *t*-test.

Table 1 shows the cyclic AMP contents of the left and right cortical areas of the rabbit brain in which different intensities of currents were applied to the left premotor cortex. The cyclic AMP contents without any addition did not vary between the polarized and non-polarized control rabbits. The cyclic AMP contents increased 7- to 34-fold upon addition of 100 $\mu$M histamine, in agreement with the previous findings showing marked cyclic AMP accumulation by histamine in rabbit cortical slices (8-10). Separate experiments showed that the effects of histamine on the elicitation of cyclic AMP accumulation was maximal at a concentration of 100 $\mu$M. In the present study, it should be noted that the mean value of histamine-elicited accumulation of cyclic AMP was decreased in the polarized or contralateral homotopic cortical area, although one-way analysis of variance revealed no

significant differences. On the other hand, when Student's *t*-test was applied by assuming an independent population, a significant decrease in the contralateral cortex occurred at 1.0 $\mu$A, whereas it occurred in the polarized cortex at 10.0 and 30.0 $\mu$A. Because of the decreases, the regional dominance in the cyclic AMP accumulation was observed in the three cases of anodal polarization, as shown in Table 1. At 0.3 $\mu$A, the cyclic AMP accumulation was practically unchanged in both cortical areas. These results indicate that repeated polarization exerts an inhibitory effect on cyclic AMP generation, and that the effect is not restricted within the cortex near the polarized point.

Table 1 also shows the effects of the $H_2$-receptor antagonist cimetidine on the elicitation of cyclic AMP accumulation by histamine; the cyclic AMP accumulation elicited by 100 $\mu$M histamine was almost completely inhibited by 100 $\mu$M cimetidine. In several species of animals, it has been shown that histamine elicited cyclic AMP accumulation through both $H_1$- and $H_2$-receptors (8, 16). In rabbit cortical slices, however, histamine has been suggested to elicit cyclic AMP accumulation solely through $H_2$-receptors (17). Taken together, the present results suggest that repeated polarization produces regional dominance in $H_2$-receptor-mediated generation of cyclic AMP due to its decrease in the cortex, which is dependent on the intensity of polarizing currents.

In rabbits, a current intensity of one to several $\mu$A has been shown to be most effective for achieving of stable peripheral motor manifestations, such as flexions of the forelimb (18). A more recent study has also revealed that rabbits showed increased motor activity after receiving unilateral repeated polarization with 1.0 $\mu$A (13). These findings suggest that lower and higher current intensities exert rather adverse effects on motor behavior. On the other hand, regional dominance in histamine-elicited accumulation of cyclic AMP has been demonstrated in the polarized cortex of rabbits showing dominant forelimb flexions after unilateral polarization (12), whereas the effects of the polarizing currents on cyclic AMP accumulation have been reported to be complex in rats (11, 19). The results of the present study indicate, however, that cyclic AMP accumulation was invariably greater in the polarized cortical area than in the contralateral cortical area when 1.0 $\mu$A was applied. Thus, it is likely that the regional dominance in cyclic AMP accumulation plays a role in the establishment of peripheral motor manifestations, for which a higher activity of cyclic AMP generation may be prerequisite.

To summarize, repeated anodal polarization of the premotor cortex of rabbits altered *in vitro* cyclic AMP accumulation through histamine $H_2$-receptors, depending on the intensity of polarizing currents. The regional dominance in cyclic AMP generation is suggested to form the neurochemical basis of the characteristic motor behavior induced by anodal polarization.

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