Reversible depression of spontaneous brain electrical activity induced by actinomycin D and 7-aminoactinomycin D in rats

E. Uezu, Y. Takada and N. Mishima

Department of Physiology, Okayama University Medical School, Okayama 700 (Japan), and Department of Biological Science, Faculty of Pharmacology, Tokushima Bunri University, Tokushima 770 (Japan), 28 July 1981

Summary. Various types of actinomycin (C,D,S_2, I and V) and 7-amino-analogue of actinomycin D were injected into the right lateral ventricle of the brain through a chronically implanted cannula. In rats but not in mice actinomycin D, actinomycin S_2 and 7-aminoactinomycin D caused depression of EEG, while cardiac and respiratory activity were maintained. This effect of the EEG was reversible and a normal EEG pattern reappeared at least 3 h after administration.

Actinomycin D (AcD) is well known as antitumor agent. The inhibiting action of the agent on DNA-dependent RNA synthesis has been well established, and it has often been used in the study of the releationship between RNA synthesis and animal behaviors. In sleep studies, we found that AcD caused a transient depression of the cortical EEG[1], but actinomycin S_3 (AcS_3), which is different from AcD in only 1 amino acid component[2], was found to be inactive[3].

In this study, we compared the effects of several other actinomycins in the rats to clarify the mechanism of the effect of AcD on the EEG.

Mice are preferred when studying the role of RNA synthesis in the memory or learning process[4]. It appeared important to examine the effects of the inhibitors on the mice EEG. AcD and actinomycin S_2 (AcS_2) were obtained from Sigma Chemical Co. and Calbiochem. Actinomycin C, I and V were obtained from Sigma Chemical Co. and P-L Biochemical Inc. 7-aminoactinomycin D (7-aminoAcD) was purchased from Calbiochem-Behring Co. Male Wistar strain rats weighing 250 g, and ddY strain mice weighing 25 g were used. As described in an earlier report[5], a cannula was chronically implanted into the right lateral ventricle of the brain in the rats and mice under anesthesia with Isozol (thiamylal sodium, 50 mg/kg, i.p.). Stainless-steel screw electrodes were placed in the cranial bone over the frontal and occipital cortices for longterm EEG recording. Electrodes were also inserted in both hippocampus and the right reticular formation in the midbrain for recording of the EEG in rats.

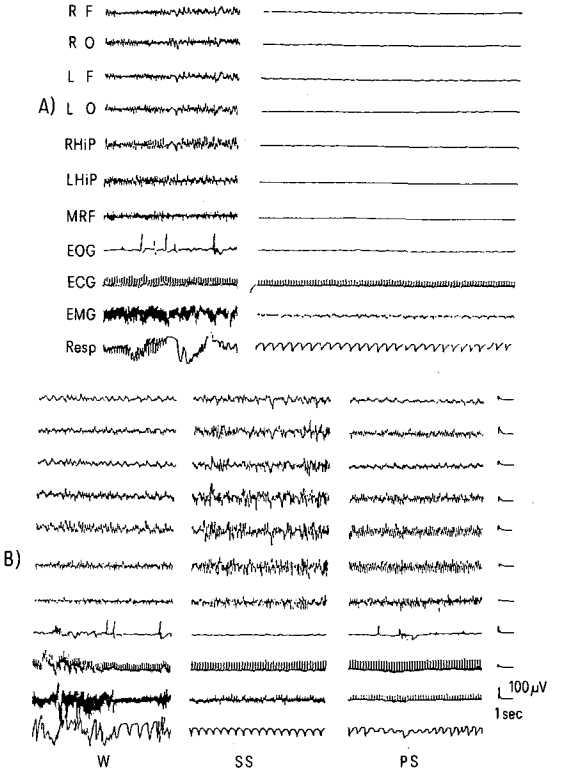
Electrodes of stainless-steel wires were used for recording an electrooculogram (EOG) and an electromyogram (EMG) of the neck muscle. The electrodes for EOG recording were inserted into anterior and posterior site of the eyeball from the outside of the orbita according to Matsumoto et al.[6].

One week after recovery from the surgery, the animals were subjected to polygraphic recording of the EEG, EOG, EMG, electrocardiogram (ECG) and respiratory activity (Resp). After taking a 2-h base line recording, one of the actinomycins dissolved in physiological saline was slowly injected in a volume of 0.25 ml/kg over 2 min through the cannula. As a control experiment, physiological saline solution was injected. The animals were recorded in free moving conditions. 20 animals were used to test each actinomycin.

In rats AcD at a dose of 0.1 mg/kg caused reversible depression of the all EEG leads for 10–30 min, while cardiac and respiratory activity were maintained in all cases as demonstrated in the figure. Reduction of the amplitude of EMG was also seen. The effects were reversible and normal signs of sleep-waking state appeared within 3 h after administration (fig., B). At a dose of 0.25 mg/kg of AcD, similar effects were seen. But, with the higher dose, half of animals also showed tardy respiratory movements, the cessation of respiration was succeeded by the stop of the cardiac activity within 30 min after administration. When the respiration was maintained by manually artificial respiration, the EEG depression continued for 30 min and then started to return to control levels. AcD of 0.05 mg/kg did not cause depression of EEG nor spike activity for at least 24 h after the administration.

AcS_2, which was thought to be a substance identical with AcD[2], showed the same effects as AcD.

7-aminoAcD, which had the same amino acid chain as AcD but is different from AcD in its chromophore structure[7], also caused transient depression of cortical and hippocampal EEG in a dose of 0.2 mg/kg. It was less effective than AcD. Further 7-aminoAcD induced generalized seizure discharges initiated by hippocampal spikes without convulsion and following depression of EEG in some rats.



Effects of actinomycin D on the EEG and EMG in a rat. Actinomycin D (0.1 mg/kg in a volume of 0.25 ml/kg) was administered into the right lateral ventricle of the brain in the rat weighing 250 g. *A* Control recording under the pretreated condition (left) and a recording at 5 min after administration (right). R, right; L, left; F, frontal cortex; O, occipital cortex; Hip, hippocampus; MRF, right midbrain reticular formation; EOG, electrooculogram; EMG, electromyogram; ECG, electrocardiogram; Resp., respiratory movements. *B* The EEG pattern recovered from the suppression by AcD. Normal signs of wakefulness (W), slow wave sleep (SS) and paradoxical sleep (PS) appeared within 3 h after administration.

Other actinomycins (I, V and C), which differed from AcD in the amino acid arrangement in the cyclic penta-peptide lacton rings but which were thought to inhibit RNA synthesis in a manner similar to AcD, did not affect the EEG even at higher dose (0.5 mg/kg). In mice, AcD of 10 µg and 7-aminoAcD of 10 µg (per mouse) caused neither disappearance of EEG nor spike activity. The effects of AcD and 7-aminoAcD seem species specific.

The findings suggest that the effects of AcD and 7-aminoAcD on the EEG are due to neither nonspecific action involved by injection of the agents nor inhibition of RNA synthesis. Such effects may be related to their direct action on the molecular structure of the neuronal membrane. The agents may also play a role as antagonists to known neurotransmitters. The sites of peptide lacton rings seem to be important for the appearance of the effect on the EEG, although the mechanism of the action is not yet understood.

1 Matsumoto, J., Morita, Y., Sano, A., Ishikawa, N., Seno, H., and Uezu, E., in: Integrative Control Functions of the Brain, vol. 1, p. 400. Eds M. Ito, N. Tsukahara, K. Kubota and K. Yagi. Kodansha/Elsevier, Tokyo and Amsterdam 1978.
2 Furukawa, M., Inoue, A., Asano, A., and Kawamata, J., J. Antibiot. *21* (1968) 568.
3 Uezu, E., Sano, A., and Matsumoto, J., Sleep Res. *8* (1979) 86.
4 Mishima, N., and Hashimoto, T., Jap. J. Physiol. *27* (1977) 267.
5 Uezu, E., Sano, A., and Matsumoto, J., Tokushima J. exp. Med. *28* (1981) 9.
6 Matsumoto, J., Nishisho, T., Sadahiro, T., and Miyoshi, M., Proc. Japan Acad. *43* (1967) 62.
7 Sangupta, S.K., Tiner, S.K., Lagaraus, H., Broun, B.L., and Modest, E.J., J. med. Chem. *18* (1975) 1175.

Down's syndrome: permeability of the erythrocyte membrane for spin-labeled non-electrolytes[1]

M. Koter, J. Kędziora[2], G. Bartosz and W. Leyko

Department of Biophysics, Institute of Biochemistry and Biophysics, University of Łódź, PL-90-237 Łódź (Poland),

17 August 1981

Summary. A decreased rate of membrane transport of a hydrophobic non-electrolyte TEMPO was found. There were no significant changes in that of a hydrophilic non-electrolyte TEMPOL in erythrocytes of patients with trisomy 21. Changes of the same direction in erythrocyte membrane permeability were found to occur during the intravascular erythrocyte aging.

Considerable evidence indicates that the genetic anomaly of trisomy 21 brings about multiple structural and metabolic disturbances at the cellular level[3,4]. The red blood cell, due to its accessibility and simplicity, has been a subject of many studies in this respect[4,5]. These studies have demonstrated alterations in the ultrastructure and functional properties of the red cell membrane, including changes in the permeability for some amino acids[4] and an abnormal pattern of osmotic fragility[5]. Our recent studies have demonstrated that the ultrastructural investigations on the erythrocyte membrane in Down's syndrome reveal irregularities typical of senescent erythrocytes in normal donors[6]. Spin label studies have pointed to alterations in the structure of membrane proteins[7] similar to those found during cell aging[8]. As the aging of bovine red cells in vivo involves characteristic changes in membrane permeability for spin-labeled non-electrolytes[8], examination of this parameter in erythrocytes from patients with Down's syndrome and comparison with changes observed during intravascular aging of human red blood cells seemed to be of interst.

Material and methods. Erythrocytes of 6 healthy donors were separated according to age using the method of Murphy[9]. Five 'layers' of cells (each making up 20% of the total cell volume) were to be differentiated in the centrifuge tube. The cells of 3 layers were used for this study: the top cells (youngest and lightest), the middle density cells, and the lowest cells (oldest and of greatest density). They were withdrawn and washed 4 times with phosphate-buffered saline. Transport of spin-labeled non-electrolytes 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) across the erythrocyte membrane was studied by the ESR method[10]. Briefly, a small amount of a stock solution of a spin label was added to a red cell suspension of hematocrit 0.50. The initial spin label concentration in the suspension was 100 µM. The sample was mounted in the cavity of an ESR spectrometer and the rate of decay of the ESR signal of a label was monitored at ambient temperature of 21 ± 1 °C. Upon entering the cell interior the labels were reduced to non-paramagnetic derivatives. Studies of kinetics of reduction of the spin labels in question by cell suspensions and hemolysates as well as effects of membrane-modifying agents on this reduction demonstrated that the rate of decay of the ESR signals of the spin labels from erythrocyte

Table 1. Permeation constants of TEMPO and TEMPOL into different age fractions of human erythrocytes (mean ± SD, n = 6); Y, youngest; M, medium; O, oldest cells

Erythrocytes	$k/min^{-1}/\times 10^2$ TEMPO	TEMPOL
Y	2.02 ± 0.25	0.83 ± 0.18
M	1.73 ± 0.15*	0.78 ± 0.12
O	1.49 ± 0.09**	0.70 ± 0.19

Statistical significance of differences with respect to youngest cells: * $p < 0.05$, ** $p < 0.01$ (Student's t-test).

Table 2. Penetration constants of TEMPO and TEMPOL into control and Down's syndrome erythrocytes (mean ± SD, n = 8 and n = 10, respectively)

Erythrocytes	$k/min^{-1}/\times 10^2$ TEMPO	TEMPOL
Control	1.75 ± 0.39	0.75 ± 0.31
Down's syndrome	1.21 ± 0.32	0.71 ± 0.18
Statistical significance of difference	$p < 0.01$	NS