Acute EEG Changes in Rats by Brainstem Ischemia and Its Dopaminergic Involvement

Yoshiki MIURA, Tsugutaka ITO and Toshiaki KADOKAWA

Department of Pharmacology, Research Laboratories, Dainippon Pharmaceutical Co., Ltd.,
33-94 Enoki-cho, Suita, Osaka 564, Japan

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Abstract—An acute animal model with irreversible ischemia in the pons (Pons Ischemic Rat, PIR) was developed by two placed occlusions of the basilar artery. PIR showed decerebrate syndromes. Electroencephalograms (EEGs) in d-tubocurarine-immobilized PIR showed inclusion of obviously higher amplitude slower waves in the cortex but similar \( \theta \) waves in the hippocampus, as compared with those in intact rats. Spontaneous cortical EEGs in PIR were desynchronized by pinching of hind limbs. From the frequency analysis, it was found that this cortical EEG alterations were composed of the decrease of \( \beta_2 \) band relative power and the increase of \( \delta-\theta_2 \) bands. Haloperidol, at the dose without effect on EEG in intact rats, dose-relatedly decreased the cortical \( \beta_2 \) band in PIR, and the potency was 100 times stronger than that in intact rats. On the other hand, the potency of atropine on the cortical \( \beta_2 \) band was almost the same in both preparations. Apomorphine, thyrotropin-releasing hormone (TRH), methamphetamine, physostigmine and amantadine dose-relatedly increased the cortical \( \beta_2 \) band in PIR, and these increasing effects, except in the case of physostigmine, were antagonized by the pretreatment of haloperidol. These results suggest that PIR is positioned as an animal model with the moderately lowered consciousness level intensively corresponding to semi coma in patients, and the dopaminergic system plays an important role rather than the cholinergic system in PIR.

Disturbances of consciousness such as coma and stupor, sometimes encountered in stroke and concussion, are mainly due to disfunction of the brainstem or diencephalon. Especially, local ischemia or hemorrhage of the brainstem leads to such disturbances. For example, Plum and Posner (1) have demonstrated from post-mortem histological examinations of patients with disturbed consciousness that local damage in the areas of the brainstem to the hypothalamus easily induces the disturbance, while in the case of the cerebral cortex, wide spread damage is necessary for the production of such a state. Recently, Fukuda et al. (2) reported that compression by balloon of the brainstem in cats, while inducing a reversible ischemia in that region, leads to coma-like behavior and a flatter of the EEG, which are improved by thyrotropin-releasing hormone (TRH). Meclofenoxate and cytidine 5’-diphosphate choline (CDP-choline) which are involved in cholinergic pathways in the central nervous system (3, 4) have been used in the therapy of coma (5, 6). Recently, however, TRH was also found to improve refractory semi coma and stupor (2). TRH, unlike meclofenoxate and CDP-choline, has been suggested to affect the central monoamine system (7, 8), especially the dopamine pathway (9, 10). Thus, at the present time, it remains obscure whether a cholinergic or dopaminergic mechanism is more intensively involved in the disturbed consciousness. In the present study, therefore, we paid attention to the pontine region and developed an animal model with irreversible ischemia in the pons caused by two placed occlusions of the basilar artery (Pons Ischemic Rat, PIR). Using this model, effects of drugs affecting
the central dopaminergic or cholinergic system were examined on spontaneous electroencephalograms (EEGs) analyzed with a Fast Fourier Transformation (FFT) routine. A preliminary report of these findings has been published previously (11).

Materials and Methods

Male Wistar rats, weighing 300–400 g, were used. 5 animals were housed in each group and given ad libitum access to both food and water until the experiment.

Preparation of PIR: Under anesthetizing with ether, rats were fixed on the back. After the trachea and esophagus were exposed, the trachea was cannulated, and the esophagus was cut between two placed ligations. After the occipital bone was exposed by scraping of the adhesive muscle, a drilled opening (diameter: 5 mm) was made in the upper part of the bone, so the basilar artery was visible under the dura matter. The dura matter was cut with a needle along the basilar artery, and the upper and lower parts of the artery (distance: 5 mm) exposed were cauterized using a coagulator of bipolar pincette electrodes (Fig. 1.). A sham operation was performed by only cutting the dura matter. In some experiments, general behaviors in PIR were observed after the operation. Following recovery from anesthesia, the rat showed decerebrate syndromes such as dilation of pupils, abnormal extension of fore and hind limbs, and irregular breathing.

Fig. 1. (A): The operative field. (B): Photograph of an ischemic area, showing unstained part after i.v. injection of methylene blue.
but no disturbance of reflexive ocular motility.

EEG recording: Under ether anesthesia, the rat was fixed on a stereotaxic apparatus (Narishige type), immobilized by d-tubocurarine chloride (1 mg/kg, i.m.) and artificially ventilated at a rate of 60 strokes/min. Silver ball electrodes were placed on the exposed right motor cortex (neocortical area, 3–4) according to the atlas of Krieg (12), and a concentric bipolar electrode, insulated except for the tip, was inserted into the hippocampus (A, 4.2; L, 2.0; H, 2.0) according to the atlas of König and Klippel (13). A reference electrode was placed in the neck muscle. EEG recordings were performed bipolarly on an ink-writing oscillograph (Nihon Kohden RJG-3006, high cut: 100 Hz and low cut: 0.01 sec in the cortex and 0.1 sec in the hippocampus). After completion of surgical procedures, anesthesia was discontinued, and EEG recordings were begun 1 hr later. All wound edges and pressure points were infiltrated with repeated injections of procaine HCl. Injection of d-tubocurarine was repeated as necessary during the course of the experiments. Exposed neural tissues were covered with warm liquid paraffin, and body temperature was maintained constant using an infrared lamp (36.5±0.5°C). Electrocardiogram (lead II) was also monitored concomitantly.

EEG frequency analysis: Cortical and hippocampal EEGs were recorded on an ink-writing recorder and simultaneously on FM magnetic tape for subsequent EEG analysis. Using a data analyzer (Nihon Kohden ATAC 450 system), power spectral analysis of EEG was performed on each epoch (4 sec) using a Fast Fourier Transformation (FFT) routine. Fifteen consecutive epochs were digitized at a sampling rate of 100 Hz and stored on the memory parts. Then the frequency bands explored were: delta (1.5–3.25 Hz), theta1 (3.5–5.75 Hz), theta2 (6.0–7.75 Hz), alpha1 (8.0–9.75 Hz), alpha2 (10.0–11.75 Hz) beta1 (12.0–19.75 Hz) and beta2 (20–40 Hz), and these relative powers were displayed on an X–Y plotter.

Determination of ischemic area in the brain: After the experiment, the ischemic area of the brain was determined by staining of dye. Namely, saturated methylene blue solution (3 ml/animal) was injected into the femoral vein. Several min later, the animal was sacrificed. The brain excised was immersed in 10% formalin for 2–3 hr and then cut longitudinally. In the basilar artery occluded rats (PIR), there was the unstained ischemic area in the pons and its neighboring regions (König and Klippel’s atlas; A: 1.0–P: 1.0, L: 0–2.5, H: −2.0–−4.5) (Fig. 1). On the other hand, the entire brain was stained in the intact and sham-operated animals.

Drugs: The drugs used in this experiment were apomorphine hydrochloride (Macfarlan Smith), TRH (Peptide Institute, Inc.), methamphetamine hydrochloride (Dainippon), amantadine hydrochloride (Nakarai), haloperidol (Dainippon), physostigmine sulfate (Merck) and atropine sulfate (Merck). All drugs, except haloperidol, were dissolved in saline, and haloperidol was used in commercial injectable form. Injection volume was always 1 ml/kg body weight. After EEGs had remained stable over 15–30 min, drugs were administered i.v. through a cannulated femoral vein for a 1 min period.

Statistics: Statistical significances of relative power in each frequency band were determined using the Student’s t-test.

Results

Characteristic EEG patterns in PIR: Spontaneous cortical EEG patterns in PIR were different from those in intact and sham-operated rats. Namely, cortical EEGs in PIR showed higher amplitude and slower waves than those in intact rats over 3 hr after the recording (Fig. 2). On the other hand, hippocampal EEGs were similar to those in intact rats. The cortical EEG patterns in PIR were changed to low amplitude fast waves by pinching of the hind limbs (Fig. 3). EEG frequency analysis indicated that the relative powers in δ–θ2 and β2 bands of cortical EEGs in PIR were 35% larger and 30% smaller than those in intact rats, respectively, while in hippocampal EEGs of PIR, the θ2 band was increased and the β band decreased (Fig. 4). These frequency patterns were maintained for over 3 hr. Sham-operated rats showed the similar EEGs to those of intact rats. Namely, cortical EEG in both preparations showed the low amplitude fast wave with the relative
Effect of haloperidol and atropine on cortical EEG in PIR: Haloperidol, a dopamine antagonist, at the low dose of 7.9 μg/kg changed cortical EEG in PIR to higher amplitude and slower waves which lasted for over 1 hr, while in intact rats, this drug affected cortical EEG only at high dose of 0.5 mg/kg (Fig. 5). When the relative power 10 min after injection was compared with the pre-injection value, haloperidol dose-relatedly reduced the cortical $\beta_2$ band in both preparations. The potency of haloperidol in PIR...
was 100 times stronger than that in intact rat. Atropine, a muscarinic antagonist, showed drowsy patterns in both preparations. However, differing from the case of haloperidol, the potency of atropine on the cortical \( \beta_2 \) band was almost the same in both preparations.

Effect of central nervous system (CNS) analeptics on cortical EEG in PIR: TRH (0.5–2.0 mg/kg, i.v.) dose-relatedly increased the cortical \( \beta_2 \) band and decreased \( \delta-\theta_2 \) bands in PIR (Fig. 6), the effect being transient (5–10 min). Methamphetamine (0.25–0.5 mg/kg), apomorphine (0.125–0.5 mg/kg), amantadine (5–20 mg/kg) and physostigmine (0.025–0.1 mg/kg) dose-relatedly increased the cortical \( \beta_2 \) band for over 20–40 min.

Effect of CNS analeptics on haloperidol-treated cortical EEGs in PIR: Effects of analeptics at doses which caused 10–25% increase in the cortical \( \beta_2 \) band were examined 10 min after the pre-treatment of haloperidol (0.5 mg/kg). Apomorphine (0.5 mg/kg), TRH (1.0 mg/kg), methamphetamine (0.25 mg/kg) and amantadine (10 mg/kg) showed little change in the cortical \( \beta_2 \) band over 30 min under the pre-treatment of haloperidol (Fig. 7). However, under the same conditions, physostigmine (0.05 mg/kg) markedly increased the cortical \( \beta_2 \) band, although this effect was somewhat weaker than that with non-treatment of haloperidol (Fig. 7).

Discussion

PIR has the following characteristics: Firstly, spontaneous EEG patterns in PIR showed high amplitude slow waves in the cortex and slow regular rhythm in the hippocampus, i.e., the dissociation between EEGs in the cortex and hippocampus. Additionally, the cortical EEGs in PIR differed from those in cereau isolé rats showing low amplitude slow waves sometimes followed by spindle bursts (14–16). Secondly, the decerebrate syndromes such as rigidity, dilation of pupils and irregular breathing observed in PIR appear to correspond to those in patients with the lowered consciousness (17) and in the cereau isolé preparation which has been suggested to reflect the

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**Fig. 5.** Effects of haloperidol and atropine on the \( \beta_2 \) band of cortical EEG in PIR. Left and right figures show the relative power of the \( \beta_2 \) band 5 min after the injections of haloperidol and atropine, respectively. Each point represents the mean±S.E.M. of the relative power, subtracted the preinjection value from the value observed 5 min after the injection, in 4-5 animals. Open circle (○) is intact rats, and closed circle (●) is PIR.
coma state (14). Thirdly, local ischemia within the brainstem, especially that restricted to the pons and its neighbouring areas in PIR, at least in part corresponds to the ischemia due to infarction of the central arterial system via the basilar artery in man, for example, pontine syndromes (17). Fourthly, cortical EEGs in PIR were easily desynchronized by pinching of the hind limbs, indicating that the activating system of the ascending brainstem reticular formation was not markedly impaired in PIR. Finally, TRH and amantadine which have been demonstrated to improve refractory semi-coma and stupor in man (5, 6) increased the $\beta_2$ relative power in the cortical EEGs in PIR. Therefore, it is suggested that PIR is an animal model with a moderately lowered consciousness level.

Haloperidol at the extremely low dose of 7.9 $\mu$g/kg decreased the $\beta_2$ relative power of cortical EEGs in PIR. The effect of haloperidol in PIR was found to be approximately 100 times stronger than that in intact rats. On the other hand, the potency of atropine was similar in both preparations. Namely, atropine at the doses of 0.5 mg/kg and 1.0 mg/kg decreased the relative power of the cortical $\beta_2$ band in PIR and intact rats. Haloperidol and atropine have been suggested to block dopaminergic and muscarinic receptors in the CNS, respectively (18, 19). Accordingly, it is suggested that under the ischemic condition of the pons and its neighbouring areas, dopaminergic receptor blockade plays more important roles than muscarinic receptor
blockade in regulation of cortical EEGs.

TRH, amantadine, apomorphine and methamphetamine have been demonstrated to activate the central dopaminergic systems (9, 20–22). For example, TRH, amantadine and methamphetamine enhance the release of dopamine from the presynaptic terminals (23–25). Amantadine also inhibits uptake of dopamine (26). Accordingly, these drugs may be expected to lead to cortical EEG desynchronization. In the present study, cortical $\beta_2$ band relative power in PIR was found to be increased by relatively low doses of TRH, amantadine, apomorphine and methamphetamine. Furthermore, haloperidol almost completely antagonized the cortical EEG desynchronization induced by these four drugs in PIR. From these results, it is assumed that dopaminergic receptor activation is involved in improving of the lowered consciousness level. However, the brain site related to dopaminergic receptor activation by the drugs examined in this study remains obscure. In the brain, the two main dopaminergic pathways are known, i.e., the neostriatal pathway originating from the cell bodies in the substantia nigra and the mesolimbic pathway from cell bodies in the ventral tegmental area (VTA) terminating to the nucleus accumbens and olfactory tuberculum (27). Especially, the latter is of great importance in mediation of the locomotor activity (21, 28). TRH, methamphetamine and apomorphine have been reported to increase locomotor activity.

Fig. 7. Effect of CNS analeptics on the cortical $\beta_2$ band in PIR with and without haloperidol. White columns (□) show $J$ relative power in nontreated PIR, and dotted columns (■) show that in haloperidol (0.5 mg/kg) treated PIR. Each column represents the mean±S.E.M. of $J$ relative power, subtracted the preinjection value ($J_2$ relative power) from the value observed 5 min after the injection of each drug in 4–5 animals, and in the case of haloperidol pretreatment, subtracted the value observed 10 min after the injection of haloperidol from the value observed 5 min after the subsequent injection of each drug. Abbreviations: Phy, physostigmine; Apo, apomorphine; TRH, thyrotropin-releasing hormone; MAP, methamphetamine; Ama, amantadine and Hal, haloperidol. Differences that are statistically significant from the value in the saline control: *P<0.05, **P<0.01; from the value in haloperidol only: #P<0.01 (Student's t-test).
through the VTA-accumbens pathway (9, 21). Thus, the positive relationship between increase of locomotor activity and cortical EEG desynchronization may be expected; however, at the present time, there is no evidence with the functional changes in the VTA-accumbens pathway possibly produced following the ischemia of the pons and its neighbouring areas. TRH has also been suggested to activate the cholinergic systems in the CNS in addition to the activation of the dopaminergic systems (29, 30). In this experiment, TRH and physostigmine increased the relative power of the cortical $\beta_2$ band in PIR. However, haloperidol completely antagonized the cortical EEG desynchronization induced by TRH, but not by physostigmine, suggesting that a cholinergic component of the TRH action contributes little to its desynchronization in PIR.

In conclusion, the focal ischemia in the pons and its neighbouring areas, induced by two placed occlusions of the basilar artery, was technically feasible and its consequence almost consistent. This ischemic model, PIR, shows acute deterioration in behavior and EEG at least in part similar to clinical stages such as the disturbance in consciousness induced by infarction of the brainstem, especially the pons. Furthermore, it is suggested that the dopaminergic system plays an important role in regulating the cortical EEGs in PIR.

References
1 Plum, F. and Posner, J.B.: The Diagnosis of Stupor and Coma, 2nd Ed., p. 2–25, F.A. Davis Comp., Philadelphia (1975)
2 Fukuda, N., Saji, Y. and Nagawa, Y.: Behavioral and EEG alterations with brainstem compression and effect of thyrotropin-releasing hormone (TRH) in chronic cats. Folia Pharmacol. Japon. 75, 321–331 (1979) (Abs. in English)
3 Geogev, V.P., Chavdarov, D., Petkov, V. and Kiriiov, B.: Effects of centrofenoxine on brain acetylcholine release upon perfusion of cerebral ventricle and dynamic electrophysiological control. Acta Physiol. Pharmacol. Bulg. 5, 59–66 (1979)
4 Yasuhara, M.: Characteristic actions of CDP-choline on central nervous system. Curr. Ther. Res. 16, 346–353 (1974)
5 Branconnier, R.J.: The efficacy of the cerebral metabolic enhancers in the treatment of senile dementia. Psychopharmacologia (Berlin) 10, 212–218 (1983)
6 Spagnoli, A. and Tognoni, G.: Cerebroactive drugs. Clinical pharmacology and therapeutic role in cerebrovascular disorders. Drugs 26, 44–69 (1983)
7 Prage, A.J., Wilson, I.C., Lara, P.P., Alltop, L.B. and Breeze, G.R.: Effect of thyrotropin-releasing hormone in depression. Lancet 2, 999–1002 (1972)
8 Keller, H.J., Bartholini, G. and Pletscher, A.: Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. Nature 248, 528–530 (1974)
9 Miyamoto, M. and Nagawa, Y.: Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats Eur. J. Pharmacol. 44, 143–152 (1977)
10 Kerwin, R.W. and Pycock, C.J.: Thyrotropin-releasing hormone stimulates release of $[^3H]$-dopamine from slices of rat nucleus accumbens in vitro. Br. J. Pharmacol. 67, 323–329 (1979)
11 Miura, Y., Ito, T. and Kadokawa, T.: Electroencephalographic study in pons anemic rats and the effect of CNS analeptics. Japan. J. Pharmacol. 36, Suppl. 330P (1984)
12 Krieg, W.J.S.: Connections of the cerebral cortex. The albino rat. A topography of the cortical areas. J. Comp. Neurol. 84, 199–208 (1964)
13 König, J.F.R. and Klippel, R.A.: A stereotaxic atlas of the forebrain and lower parts of the brainstem. In The Rat Brain, Baltimore, Williams and Wilkins (1963)
14 Bremer, F.: L'activité cérébrale au cours du sommeil et de la narcose. Bull. Acad. Roy. Méd. (Belg.) 2, 68–86 (1937)
15 Kawamura, H., Nakamura, Y. and Tokizane, T.: Effect of acute brainstem lesions on the electrical activities of the limbic system and neocortex. Japan. J. Physiol. 11, 564–575 (1961)
16 Fukuda, H., Watanabe, K. and Ito, T.: Effect of lyoniol-A on decerebrate rigidity and electroencephalogram in rats. Japan. J. Pharmacol. 22, 457–465 (1972)
17 Turazzi, S. and Bricolo, A.: Acute pontine syndrome following head injury. Lancet 2, 8028–8029 (1977)
18 Seeman, P., Lee, T., Chau-Wong, M. and Wong, K.: Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261, 717–718 (1976)
19 Yamamura, H.I. and Snyder, S.H.: Muscarinic cholinergic binding in rat brain. Proc. Natl. Acad.
Ervin, G.N., Schmitz, S.A., Nemeroff, C.B. and Prage, A.J.: Thyrotropin-releasing hormone and amphetamine produce different patterns of behavioral excitation in rats. Eur. J. Pharmacol. 72, 35–43 (1981)

Kelly, P.H., Sevior, P.W. and Iversen, S.D.: Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 94, 507–519 (1975)

Svensson, T.H. and Stromberg, U.: Potentiation by amantadine hydrochloride of L-DOPA induced effects in mice. J. Pharm. Pharmacol. 22, 639–645 (1970)

Cohn, M.L., Cohn, M. and Taylor, F.H.: Thyrotropin-releasing factor (TRF) regulation of rotation in the non-lesioned rat. Brain Res. 96, 134–142 (1975)

Scatton, B., Cheramy, A., Besson, M.J. and Glowinski, J.: Increased synthesis and release of dopamine in the striatum of the rat after amantadine treatment. Eur. J. Pharmacol. 13, 131–133 (1970)

Stromberg, U., Svensson, T.H. and Waldeck, B.: On the mode of action of amantadine. J. Pharm. Pharmacol. 22, 959–961 (1970)

Fletcher, E.A. and Redfern, P.H.: The effect of amantadine on the uptake of dopamine and noradrenaline by rat brain homogenate. J. Pharm. Pharmacol. 22, 967–989 (1970)

Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Supp. 367, 1 (1971)

Pijnenburg, A.J.J., Honig, W.M.M. and Van Rossum, J.M.: Effect of antagonists upon locomotor stimulation induced by injection of dopamine and noradrenaline into the nucleus accumbens of nialamide-pretreated rats. Psychopharmacologia (Berlin) 41, 175–185 (1976)

Yarbrough, G.G.: Thyrotropin-releasing hormone and CNS cholinergic neurons. Life Sci. 33, 111–118 (1983)

Yarbrough, G.G.: TRH potentiates excitatory actions of acetylcholine on cerebral cortical neuron. Nature 263, 523–534 (1976)