SLEEP STATE AND ITS INDUCTION BY SODIUM BUTYRATE IN ACUTE "ENCEPHALE ISOLÉ" AND "ISOLATED MIDBRAIN-PONS-MEDULLA" PREPARATIONS

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In the preceding studies¹⁻³⁾, the authors showed that in the intact animal the intravenous injection of sodium butyrate induced a sleep state characterized by slow wave EEG pattern (ortho-sleep), followed by another phase of sleep (para-sleep) characterized by several features such as low-voltage fast wave EEG pattern, no activity of neck muscle and rapid eye movements. In addition, the effect of the butyrate for induction of sleep was analyzed by using cats with transections at different levels of the brain stem⁴⁻⁶⁾. The present study deals with the sleep states and their induction by sodium butyrate in the acute "encéphale isolé" preparation⁷⁾.

METHODS

Thirty-six cats, 2.5 to 4 kg body weight, were used. In 21 cats, electrodes for EEG recording were implanted in the brain. Method of the preliminary operation for electrode implantation was similar to that reported in the previous study³⁾. After 1 to 2 weeks, control observation of polygraphic recordings was made, and then the brain stem was transected between the medulla and spinal cord at C₁ level ("encéphale isolé" preparation) under the ether anesthesia given via tracheal cannula. In 15 cats, under similar anesthesia the brain was transected at the precollicular level with forebrain removed, and the brain stem was, in addition, transected at the spinal cord C₁ level ("isolated midbrain-pons-medulla" preparation ("IMPM" preparation)). Artificial respiration was required immediately after the spinal transection. Methods of the brain stem transection, electrode implantation and polygraphic recordings, and also of injection of compounds (sodium butyrate, Ringer's solution and glucose) were similar to those reported previously¹⁻³⁾.

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RESULTS

1) *EEG pattern and other activities in the course of sleep-wakefulness.*

In the "encéphale isolé" and "IMPM" preparations with artificial respiration, polygraphic recordings were continued for 1 to 2 days postoperatively. Ringer's solution or glucose was injected intravenously or intraperitoneally at intervals of 2 to 3 hours during observation period.

(1) "*Encéphale isolé*" preparation. In the "encéphale isolé" preparation, polygraphic pattern was divided into 3 patterns as in the intact animal. When the animal showed purposeful movement of eyes, EEGs showed low-voltage fast waves in the neocortex and nucleus reticularis pontis caudalis (NRPC), and theta waves in the hippocampus (FIG. 1A). The neck muscle activity showed tonic discharges. This pattern was identical to that of awake in the intact animal. When the animal was behaviorally silent, the EEGs in the neocortex and NRPC changed to slow waves and spindle bursts (FIG. 1B). Theta waves in the hippocampus changes to slow-wave pattern with spikes. These polygraphic changes were similar to those of the ortho-sleep in the intact animal. After an appropriate period of ortho-sleep, the neocortical EEG changed to low-voltage fast waves, and theta waves (4-6 c/s) reappeared in the hippocampus; while the preparation was behaviorally silent (FIG. 1C). At the same time, outbursts of spike activity (4-8 c/s) appeared in the NRPC accompanied by rapid eye movements. The neck EMG activity continued to show tonic discharges. These polygraphic changes were similar to those of para-sleep in the intact animal except for the neck EMG activity which disappeared during such state. This state might be interpreted to be para-sleep state in this preparation. Usually, such state of para-sleep with a 3- to 10-min duration occurred at intervals of 1 to 3 hours after 10- to 20-hours postoperatively.

(2) "*IMPM*" preparation. In the "IMPM" preparation, two different states were distinguished polygraphically as in the mesencephalic cat⁶). The first state was marked by low-voltage fast waves in the NRPC and no movement of eyes (FIG. 2A). The tonic discharges of neck muscle was sustained with little fluctuation. Another state was distinguished polygraphically from the former state by outbursts of spike activity (4-8 c/s) within the NRPC and rapid eye movements (FIG. 2B, C). These polygraphic changes were similar to those of para-sleep state in the mesencephalic cat⁶). This state might be interpreted to be para-sleep state in the "IMPM" preparation. Usually, the para-sleep state with a 1- to 6-min duration occurred at intervals of 30-min to 2-hours, after a recuperative period of 15 to 25 hours.

FIG. 1. Polygraphic recordings of naturally occurring (A) awake, (B) ortho-sleep and (C) para-sleep states in the "encéphale isolé" preparation. In each, from top to bottom, EEG of the nucleus reticularis pontis caudalis (L. n. RPC), hippocampus (L. HIP), anterior sigmoid gyrus (L. ANT. SIG), electromyographic activity of neck muscle (EMG), eye movement (EYE), artificial respiration (RESP(A)) and electrocardiogram (ECG) are recorded. L indicates left side. Note the outbursts of spike activity within L. n. RPC and eye movements in C during para-sleep state.

FIG. 2. Polygraphic recordings of naturally occurring (A) non-para-sleep and (B, C) para-sleep state in the "isolated midbrain-pons-medulla (IMPM)" preparation. A and B are continuous, and in B transition from non-para-sleep to para-sleep state. C is 5 min after B, and in C transition from para-sleep to non-para-sleep state. In each, from top to bottom, EEG of the N. RPC by bipolar record (L. n. RPC), monopolar record with inner core electrode (L. n. RPC (M₁)), and sheath electrode (L. n. RPC (M₂)) of a concentric bipolar electrode, neck EMG, eye movement, artificial respiration and ECG are recorded. Note the outbursts of spike activity within L. n. RPC and eye movements in B and C during para-sleep state.

2) *Effects of intravenous injection of sodium butyrate upon EEG and other activities.*

Sodium butyrate was administered intravenously to 15 "encéphale isolé" and 9 "IMPM" preparations, and its effect was tested as indicated by EEG and other activities in polygraph.

(1) "*Encéphale isolé*" preparation. In the "encéphale isolé" preparation, 4 to 20 hours after the spinal transection, injection of the butyrate or Ringer's solution as a control was begun. Before injection of the butyrate, polygraphic recordings were continued for 2 to 5 hours, and Ringer's solution was injected

FIG. 3. Polygraphic recordings after intravenous injection of sodium butyrate (1.5 mM/kg) in the "encéphale isolé" preparation (same preparation used in FIG. 1). A shows the state before injection. B, C, D and E show that of 1, 3, 10 and 30 min respectively after injection. In C, transition from ortho- to para-sleep state, and in D transition from para- to ortho-sleep state. (For abbreviations see legends to FIG. 1).

at intervals of 50 to 90 min. FIG. 3 shows an example of the sequential changes of polygraphic records after injection of the butyrate (1.5 mM (1.5 ml of 1 M solution)/kg) to the "encéphale isolé" preparation, which is the same one used for polygraphic recording in FIG. 1. Before the injection, EEGs showed low-voltage fast waves in the neocortex and NRPC, and theta waves in the hippocampus (FIG. 3A). The preparation awakened behaviorally. One min after injection, the neocortical EEG changed to slow waves and spindle bursts, and the hippocampal theta waves to slow waves with spikes (FIG. 3B). Three min later (FIG. 3C), when the preparation was behaviorally silent the neocortical EEG changed to low-voltage fast waves, and theta waves reappeared in the hippocampus. At the same time, the outbursts of spike activity appeared in the NRPC accompanied by rapid eye movements. The neck EMG continued to show tonic discharges unchanged. These phenomena are similar to those of the naturally occurring para-sleep state in the same preparation (FIG. 1C). This state continued for 7 min (FIG. 3D), then the outbursts of spike activity within NRPC and eye movements disappeared, and slow-waves and spindle bursts reappeared in the neocortex. Usually, 20 to 30 min after injection the polygraphic pattern returned to the state before injection (FIG. 3E). The same amount of butyrate (1.5 mM/kg) was injected repeatedly 5 times at intervals of 60 min to the same preparation. One-half to 1 min after injection the slow wave state (ortho-sleep) occurred for 2 to 6 min, and then the para-sleep state followed for 3 to 11 min in every test. In 76% of 59 trials in 15 "encéphale isolé" preparations, para-sleep state with a 2- to 12-min duration occurred within 10 min after injection of the butyrate (1.5-2 mM/kg); while the ortho-sleep state occurred in all trials. In control experiments, para-sleep state occurred in 6% of 30 trials for 13 preparations within 10 min after injection of Ringer's solution.

(2) "*IMPM*" preparation. In order to test the effect of sodium butyrate upon the brain stem in the absence of the precollicular forebrain and spinal cord, this compound was injected to the "IMPM" preparation. Usually, the butyrate injection was begun 4 to 5 hours after the operation. FIG. 4 shows an example of the sequential changes of polygraphs after injection of the butyrate (1.5 mM/kg). Before the injection, EEG showed low-voltage fast waves in the NRPC (FIG. 4A). One min after injection, outbursts of spike activity appeared within the NRPC accompanied by rapid eye movements (FIG. 4B). Neck EMG continued to show tonic discharges unchanged. The outbursts of spike activity within the NRPC and the eye movements were identical to those of naturally occurring para-sleep in the same preparation (FIG. 2B, C). This state continued for 7 min. When the butyrate (1.5 mM/kg) was injected 5 times at intervals of 60 min to the same preparation, para-sleep state with a 6.5- to 9-min duration appeared after a delay of 0.5- to 1-min in every test. Ringer's solution did not induce para-sleep state under the same experimental conditions. These observations indicate that para-sleep state was induced by the butyrate

FIG. 4. Polygraphic recordings after intravenous injection of sodium butyrate (1.5 mM/kg) in the "IMPM" preparation (same preparation used in FIG. 2). A shows the state before injection. B, C and D show that of 1, 7 and 30 min respectively after injection. In B, transition from non-para-sleep to para-sleep state, and in C transition from para-sleep to non-para-sleep state. (For abbreviations see legends to FIG. 1 and 2).

in the "IMPM" preparation. In 79% of 34 trials in 9 "IMPM" preparations, para-sleep state appeared within 10 min after injection of the butyrate (1.5–2 mM/kg). In control experiments, para-sleep state occurred within 10 min after injection of Ringer's solution in 13% of 28 trials in 11 preparations. The effectiveness of the butyrate for induction of para-sleep was significant as compared with that of Ringer's solution ($P < 0.01$).

Compared with the "encéphale isolé" preparation, in the "IMPM" preparation the para-sleep state appeared with a relatively short latent period after injection of the butyrate (FIG. 5). In the "encéphale isolé" preparation the

FIG. 5. Frequency histogram of the onset time of para-sleep state after intravenous injection of sodium butyrate (1.5 mM/kg) in the "encéphale isolé" (A) and "IMPM" (B) preparations. The ordinate shows number of para-sleep states, and abscissae shows the onset time of para-sleep state after injection. In each, N indicates number of total phases of para-sleep. In A, N is 45 which was obtained by 59 injections of the butyrate to 15 "encéphale isolé" preparations (effectiveness, 76%). In B, N is 27 which was obtained by 34 injections of the butyrate to 9 "IMPM" preparations (effectiveness, 79%). Mean onset time of para-sleep state is 4 min in A and 2 min in B. Difference in the mean onset time between A and B is significant ($P < 0.05$).

mean onset time of the para-sleep state after the butyrate injection was 4 min ($N=45$ in 15 preparations), while that of the "IMPM" preparation was 2 min ($N=27$ in 9 preparations). Difference in the mean onset time of the para-sleep state between the "encéphale isolé" and "IMPM" preparations was significant ($P<0.05$).

DISCUSSION

It has been known that the classical "encéphale isolé" preparation showed persistent low-voltage fast wave EEG pattern^{8,9}. Recently, it was shown that by elimination of pain or hyperventilation there is a tendency for slow wave EEG and ocular signs of sleep to appear¹⁰. The present study suggested that in the "encéphale isolé" preparation both states of ortho-sleep and para-sleep were observed; the latter phase of sleep was characterized by low-voltage fast waves in the neocortex, theta waves in the hippocampus, outbursts of spike activity within the NRPC and rapid eye movements. In addition, it was shown that intravenous injection of sodium butyrate induced the ortho-sleep state of 2-6 min duration, followed by a 3-11 min period of the para-sleep. This result indicates that sodium butyrate is capable of inducing the states of para- as well as ortho-sleep in the brain disconnected from the spinal cord. In the "IMPM" preparation, para-sleep state characterized by outbursts of spike activity within the NRPC and eye movements occurred naturally and also was induced by the butyrate. These phenomena were similar to those of mesencephalic para-sleep except for neck EMG activity which disappeared during such state^{1,6}. This observation suggests that contribution from the precollicular forebrain and spinal cord plays no essential part for production of para-sleep, and that the butyrate acts on the lower brain stem in inducing para-sleep. This finding suggests also that disappearance of neck EMG activity during para-sleep may be due to active influence from the lower brain stem.

The butyrate has been shown to exert depressant action on the central nervous system leading to slow waves and behavioral sleep; suggesting that sleep-inducing influence of the butyrate might act through a depressant action on the arousing system^{5,6,11-14}. In the "IMPM" preparation, slow waves which is recorded from the NRPC of the "encéphale isolé" preparation as well as intact animal during ortho-sleep was constantly absent, even after administration of the butyrate. In addition, in this preparation the para-sleep state appeared with a significantly short delay time after the butyrate injection compared with the "encéphale isolé" preparation. These observations may suggest that the brain stem is influenced by the precollicular forebrain for appearance of slow waves^{6,15}, and also that during ortho-sleep the forebrain exerts suppressive influence on the brain stem for producing para-sleep. The interaction between the forebrain and lower brain stem in producing both

phases of para- and ortho-sleep remains to be studied.

SUMMARY

Sleep states and their induction by sodium butyrate were studied in acute "encéphale isolé" (transection at the spinal cord C_1 level) and "IMPM" ("isolated midbrain-pons-medulla") (precollicular decerebration and transection at the spinal cord C_1 level) preparations. The naturally occurring ortho- and para-sleep states were observed in the "encéphale isolé" preparation. The intravenous administration of sodium butyrate (1.5–2 mM/kg) induced ortho-sleep state of 2- to 6-min duration, followed by a 2–12 min period of the para-sleep state. Similarly, in the "IMPM" preparation para-sleep state was observed, and also was induced by the butyrate. When the same amount of the butyrate (1.5 mM/kg) was given repeatedly at about 1 hour intervals, para-sleep state was reproducible in these preparations.

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