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Selective Activation of CCK-B Receptors Does Not Induce Sleep and Does Not Affect EEG Slow-Wave Activity and Brain Temperature in Rats[1]

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CHANG, H.-Y. AND L. KAPÁS. *Selective activation of CCK-B receptors does not induce sleep and does not affect EEG slow-wave activity and brain temperature in rats.* PHYSIOL BEHAV **62**(1) 175–179, 1997.—Systemic injections of cholecystokinin octapeptide sulfate ester (CCK-8-SE) elicit various behavioral and autonomic responses, such as increases in nonrapid-eye-movement sleep (NREMS) and hypothermia. There are two CCK receptors; both CCK-A and CCK-B receptors are stimulated by CCK-8-SE. The relative importance of the CCK-A and CCK-B receptors in the somnogenic and hypothermic effects of CCK-8-SE is not well understood. In the present experiments, we studied the effects of the selective activation of CCK-B receptors by CCK tetrapeptide (CCK-4) or nonsulfated CCK-8 (CCK-8-NS) on sleep and brain temperature (T_{br}). Rats were injected intraperitoneally with saline on the control day and with CCK-8-NS (10, 50, or 250 μg/kg) or CCK-4 (10, 50, or 250 μg/kg) on the test day 5–10 min before dark onset. Electroencephalogram, electromyogram, and T_{br} were recorded for 12 h. None of the treatments affected sleep or T_{br} significantly, with the exception of 10 μg/kg CCK-4, which transiently decreased the amount of NREMS, and 10 μg/kg CCK-8-NS, which slightly increased REMS. These results suggest that the activation of CCK-B receptors by systemic injection of CCK-4 or CCK-8-NS is not sufficient to elicit increased NREMS and hypothermia in rats. © 1997 Elsevier Science Inc.

Sleep Nonrapid-eye-movement sleep Rapid-eye-movement sleep Brain temperature EEG slow-wave activity CCK-4 CCK-8-NS CCK-B receptor Rat

CHOLECYSTOKININ (CCK) is a gastrointestinal hormone and a neurotransmitter/neuromodulator in the nervous system. CCK has a wide variety of autonomic and behavioral effects; it induces anxiety and hypolocomotion, inhibits food intake, and affects sexual behavior, memory, and thermoregulation [reviewed in (3)]. Exogenous administration of CCK octapeptide sulfate ester (CCK-8-SE) promotes physiological sleep resembling that which normally follows satiation (14); systemic injections of 10–50 μg/kg CCK-8-SE dose-dependently increase sleep in rats (9) and rabbits (10).

There are two CCK receptor subtypes. CCK-B receptors are abundant in various brain regions, such as the cerebral cortex, olfactory bulbs, hippocampus, amygdala, nucleus accumbens, and nucleus tractus solitarius [reviewed in (19)], but CCK-B receptors are also found in the periphery (e.g., on the vagus nerve) (13). CCK-A receptors are located mainly in the gastrointestinal tract; in the brain, CCK-A receptors are restricted only to a few regions such as area postrema, nucleus tractus solitarius, interpenduncular nucleus, and the posterior hypothalamus (15).

Both CCK-A and CCK-B receptors are involved in mediating the effects of CCK on the nervous system. For example, the anxiogenic (19) and febrile (18) actions of CCK are mediated by CCK-B receptors, whereas the suppressive effects of CCK on feeding are mediated by CCK-A receptors (7). The involvement of CCK-A and the CCK-B receptors in the somnogenic effects of CCK, however, remains unclear. The aim of the present study was to test the effects of CCK-B receptor activation by CCK tetrapeptide (CCK-4) and nonsulfated CCK-8 (CCK-8-NS) on spontaneous sleep in rats. CCK-8-NS and CCK-4 have similar affinities to the CCK-B receptor as CCK-8-SE, but their affinities to CCK-A receptors are 500–10,000-fold weaker than that of CCK-8-SE (19), thereby providing a useful tool for activating CCK-B receptors selectively. Our results suggest that the activation of CCK-B receptors is not sufficient to elicit sleep or

[1] Part of the present study results have been reported in abstract form (Soc. Neurosci. Abstr. 22, Part 1:147, 1996.)

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hypothermic responses. A part of the present results has been reported in abstract form (2).

MATERIALS AND METHODS

Materials

CCK-8-NS and CCK-4 (Peninsula Lab., Inc., Belmont, CA) were dissolved in isotonic NaCl solution immediately before the injections.

Animals

Male Sprague–Dawley rats (320–400 g) were implanted with chronic electrodes for cortical electroencephalogram (EEG) and nuchal electromyogram (EMG), and a thermistor for cortical brain temperature (T_{br}) recordings using combined ketamine (85 mg/kg) and xylazine (15 mg/kg) anesthesia. The EEG electrodes were placed over the frontal and parietal cortices; the thermistor was placed upon the dura over the parietal cortex. After an 8–10-day recovery period, the animals were placed into sound-attenuated individual experimental cages for adaptation to the experimental conditions. During this 6–8-day habituation period, the rats were connected to recording cables and injected with saline daily at dark onset. The animals were kept on a light-dark cycle of 12-12 h (dark onset at 2000 h) and at an ambient temperature of 26 ± 0.5°C for at least 2 weeks before surgeries, during the recovery, habituation, and the experimental periods. Water and food were available ad libitum throughout the experiment.

Recordings

EEG, EMG, and T_{br} were recorded by computer. The EMG recordings served as an aid for determining the vigilance states and were not further quantified. EEG was filtered below 0.1 and above 40 Hz, EMG was filtered below 250 and above 1000 Hz. The amplified signals were digitized at the frequency of 128 Hz for EEG, and at 2 Hz for EMG and T_{br}. Single T_{br} samples were saved on the hard disc in 10-s intervals. Online fast Fourier analysis of the EEG was also performed in 10-s intervals on 2-s segments of the EEG in 0.5 Hz bands of the 0.5–30 Hz frequency range. The vigilance states were determined offline in 10-s epochs. EEG, EMG, and T_{br} were displayed on the computer monitor in 10-s epochs, and also simultaneously in a more condensed form, in 12-min epochs. Wakefulness, nonrapid-eye-movement sleep (NREMS), and rapid-eye-movement sleep (REMS) were distinguished as described before in detail (12). Briefly, NREMS was characterized by high amplitude slow-wave EEG activity and low EMG activity; REMS was characterized by low-amplitude, fast-wave EEG activity with a regular, visible theta rhythmicity and a lack of muscle tone occasionally interrupted by muscle twitches; wakefulness was characterized by irregular, low-amplitude, fast-wave EEG activity and irregular, high EMG activity. The EEG power density values were measured in the delta (0.5–4 Hz) band for each 10-s epoch of NREMS and then averaged in 1-h time blocks. EEG delta activity during NREMS, also called slow-wave activity (SWA) is often regarded as an indicator of NREMS intensity (12).

Experimental Protocol

Six groups of rats were used. On the control day, all the animals were injected with vehicle intraperitoneally (IP). On the test day, 3 groups of rats received 3 different doses of CCK-8-NS [10, 50, and 250 μg/kg (9.4, 47.1, and 235.3 nmol/kg), $n = 7$, $n = 11$, and $n = 6$, respectively] and the other 3 groups were treated with CCK-4 [10, 50, or 250 μg/kg (16.8, 83.9, and 419.3 nmol/kg), $n = 7$, $n = 7$, and $n = 7$, respectively] IP. The order of the control and test days was counterbalanced. Saline, CCK-4, and the two lower doses of CCK-8-NS were injected in a volume of 2 ml/kg; 250 μg/kg CCK-8-NS and its control, vehicle treatment, were injected in a volume of 10 ml/kg. All the injections were done 5–10 min before dark onset. EEG, motor activity, and T_{br} recordings started at dark onset and continued for 12 h. Due to the malfunction of several thermistors, the animal numbers for T_{br} are lower (50 μg/kg CCK-8-NS, $n = 8$; 250 μg/kg CCK-8-NS, $n = 5$; 10, 50, and 250 μg/kg CCK-4, $n = 6$, $n = 4$, and $n = 6$, respectively). The recordings from the rats that had artifact contamination in their EEGs were excluded from the fast Fourier analysis. The sample size, therefore, for SWA analysis is lower than for sleep recordings (10 and 50 μg/kg CCK-8-NS, $n = 5$, $n = 8$, respectively; 10 and 50 μg/kg CCK-4, $n = 6$, $n = 5$, respectively).

Statistical Analysis

Comparisons were made between the control and experimental days by using two-way ANOVA for repeated measures and paired t-test a posteriori. Time spent in different vigilance states and the SWA were calculated in 1-h time blocks; ANOVA was performed on the 1-h time blocks across 12 h. For T_{br}, ANOVA was performed across 12 h on values averaged in 1-h intervals.

RESULTS

The lowest dose, 10 μg/kg CCK-4 had a significant effect on NREMS and T_{br} across the 12-h recording period, as indicated by ANOVA (Fig. 1; ANOVA for repeated measures, NREMS treatment effect: $F(1,6) = 11.99$, $p < 0.05$; T_{br} treatment effect: $F(1,5) = 9.42$, $p < 0.05$). Post hoc paired t-test showed a significant decrease in NREMS in Hour 4; for T_{br}, the post hoc analysis did not indicate any significant effect in any h. REMS and SWA were not affected by 10 μg/kg CCK-4. The other 2 doses, 50 and 250 μg/kg of CCK-4, did not affect NREMS, REMS, SWA, or T_{br}. CCK-8-NS did not have any significant effect on NREMS, SWA, or T_{br} at any of the tested doses (Fig. 2). Ten μg/kg CCK-8-NS, however, significantly affected REMS across the 12-h recording period [ANOVA for repeated measures, treatment effect: $F(1,6) = 27.32$, $p < 0.05$]. REMS was significantly increased in Hour 3 (paired t-test, $p < 0.05$).

DISCUSSION

Intraperitoneal injection of 10–50 μg/kg CCK-8-SE (8.7–43.7 nmol/kg) causes increased sleep during the first 1–2 h after treatment in rats (9) and rabbits (10). CCK-8-SE binds to both CCK-A and CCK-B receptors with similar affinities (20). The aim of the present experiments was to study if selective activation of CCK-B receptors is sufficient to elicit increases in sleep as observed after CCK-8-SE treatment. The affinities of CCK-4 and CCK-8-NS to the CCK-B receptors are ~10 times weaker than that of CCK-8-SE (20), but their affinities to CCK-A receptors are 500–10,000-fold less than that of CCK-8-SE (19). If the somnogenic effects of CCK-8-SE are due to the selective activation of CCK-B receptors, then 90 nmol/kg CCK-4 or CCK-8-NS would be expected to elicit similar somnogenic actions as 9 nmol/kg CCK-8-SE. In our experiments, the amount of NREMS and NREMS intensity (as indicated by SWA) were not increased after the injection of 16.8–419.3 nmol/kg CCK-4 or 9.4–235.3 nmol/kg CCK-8-NS; rather, NREMS was slightly decreased after the injection of 10 μg/kg (16.8 nmol/kg) CCK-4. Similarly, 8 μg/kg BC-264, another selective agonist of the CCK-B receptors, slightly increases wakefulness in

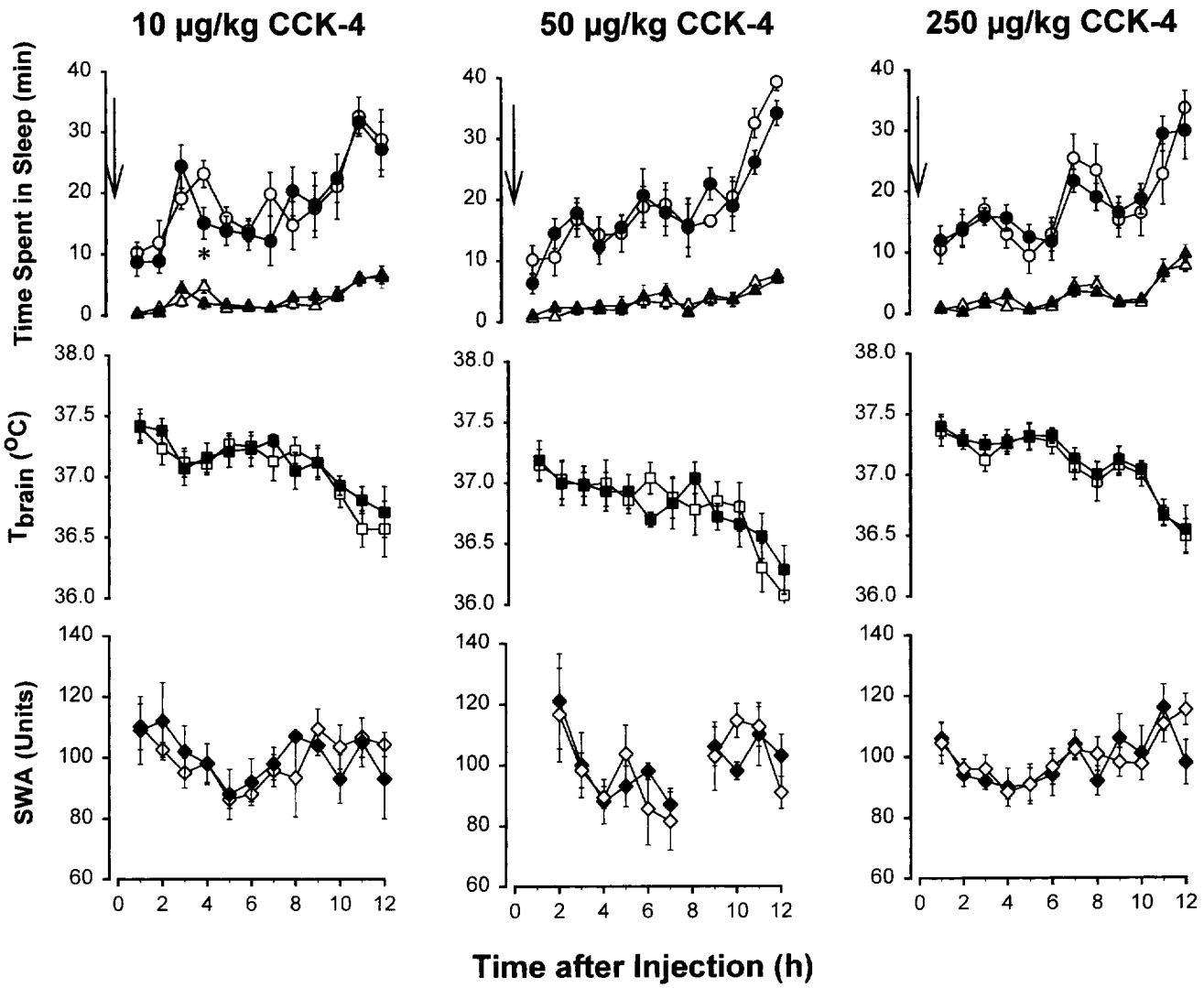

FIG. 1. The effects of cholecystokinin tetrapeptide (CCK-4) on nonrapid-eye-movement sleep (NREMS, ●; compared to vehicle ○), rapid-eye-movement sleep (REMS, ▲; compared to vehicle △), brain temperature (T_{br}, ■; compared to vehicle □), and slow-wave activity of the electroencephalogram during NREMS (SWA, ◆; compared to vehicle ◇). The data points represent means ± standard error; arrows, time of the injection; missing data points for SWA: 3 or less animals exhibited NREMS in a certain h and SWA was not calculated for that h because of the low sample size. CCK-4 did not affect any of the measured parameters with the exception of the 10 µg/kg dose, which slightly suppressed NREMS in Hour 4 [ANOVA for repeated measures, NREMS treatment effect: $F(1,6) = 11.99$, $p < 0.05$; * post hoc paired t-test, $p < 0.05$ in Hour 4; ANOVA for repeated measures, T_{br}: $F(1,5) = 9.42$, $p < 0.05$]. Sample size for 10 µg/kg dose: $n = 7$, 6, and 6 for sleep, T_{br}, and SWA, respectively; 50 µg/kg: $n = 7$, 4, and 5; 250 µg/kg: $n = 7$, 6, and 7.

rats (6) and CCK-4 induces behavioral activation in the open-field test (8). The lowest dose of CCK-8-NS induced a statistically significant increase in REMS. The biological significance of this effect is, however, unclear. The magnitude of the increase was rather small (1.4 min in 1 h) and, unlike the somnogenic effects of CCK-8-SE that occur immediately in the first h (9,10,14), it appeared in the third h after the injection. Taken together, our results indicate that the selective activation of CCK-B receptors is not sufficient to elicit somnogenic responses characteristic of CCK-8-SE. In accordance with this, preliminary data suggest that the somnogenic effects of CCK-8-SE are completely blocked by a CCK-A receptor antagonist (H.-Y. Chang and L. Kapás, unpublished observations).

In our experiments, neither CCK-8-NS nor the two higher doses of CCK-4 altered T_{br} significantly. Ten µg/kg CCK-4 had a significant effect on T_{br} as indicated by ANOVA, but the biological relevance of this effect is questionable because post hoc statistical analysis did not reveal any time point at which T_{br} significantly differed from that in the controls. These findings are consistent with previous reports that systemic injections of 100 or 200 µg/kg CCK-4 or 200 µg/kg CCK-8-NS (11) or intracerebroventricular (ICV) injection of CCK-4 and CCK-8-NS do not affect body temperature (17). In a recent study, ICV injection of CCK-8-SE induced fever-like thermoregulatory responses in female rats; these responses were inhibited by the CCK-B receptor blocker L-365,260 suggesting the involvement of CCK-B receptors in fever genesis (18). Our data suggest, however, that the stimulation of CCK-B receptors by systemic injections of CCK-B receptor agonists is not sufficient to induce fever in male rats. It is possible that, when injected systemically, CCK-4 and CCK-8-NS do not reach the brain regions that mediate the febrile ef-

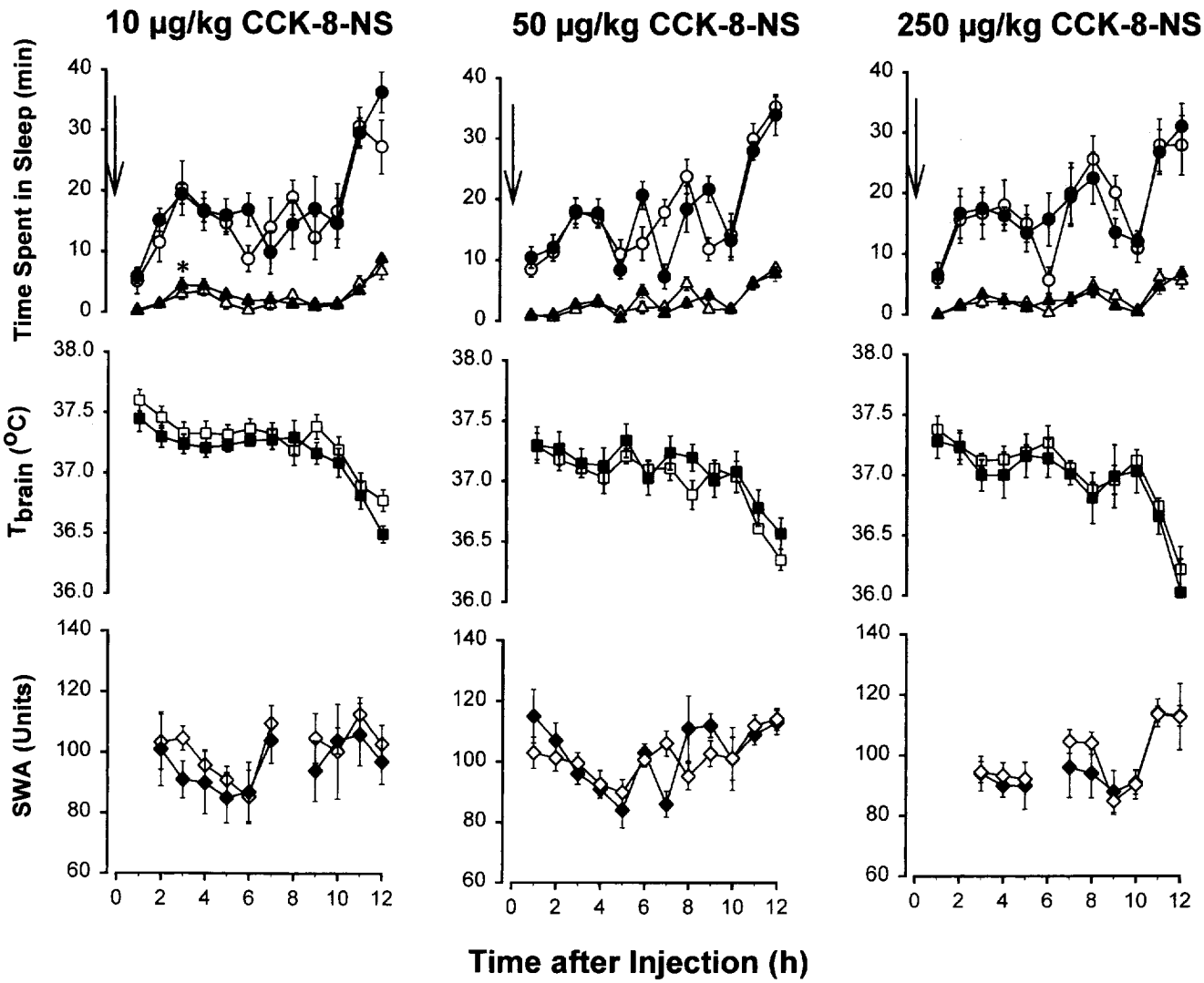

FIG. 2. The effects of nonsulfated CCK octapeptide (CCK-8-NS) on NREMS, REMS, T_{br}, and SWA. See legend to Fig. 1 for details. CCK-8-NS did not affect any of the measured parameters, with the exception of the 10 μg/kg dose that slightly increased REMS in Hour 3 [ANOVA for repeated measures, REMS treatment effect: $F(1,6) = 27.32$, $p < 0.05$; * post hoc paired t-test, $p < 0.05$ in Hour 3]. Sample size for 10 μg/kg dose: $n = 7$, 7, and 5 for sleep, T_{br}, and SWA, respectively; 50 μg/kg: $n = 11$, 8, and 8; 250 μg/kg: $n = 6$, 5, and 6.

fects of ICV-administered CCK-8-SE in sufficient amounts to initiate CCK-B receptor-mediated febrile responses.

Systemic injection of CCK-8-SE elicits the complete sequence of "satiety syndrome," including the cessation of eating, reduced exploration, social withdrawal, and sleep (1). Similar to the lack of sleep-promoting effects of CCK-B receptor agonists, the other components of the satiety syndrome cannot be elicited by the selective activation of CCK-B receptors. For example, systemic injection of CCK-4 (5) or CCK-8-NS (4) does not affect food intake and exploratory behavior. Furthermore, central injection of physiological doses of CCK-8-SE does not induce sleep (10,16). These results indirectly suggest that CCK may act on the periphery primarily on CCK-A receptors or simultaneously on both CCK-A and CCK-B receptors to elicit the complete sequence of the satiety syndrome, including sleep.

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