Pharmacology Biochemistry & Behavior, Vol. 38, pp. 545–548. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Effect of a Highly Selective Central CCK-B Receptor Agonist: BC-264 on Rat Sleep

ZARA DE SAINT HILAIRE,*†[1] BERNARD P. ROQUES‡ AND STYLIANOS NICOLAÏDIS*

**Neurobiologie des Régulations, CNRS UA 637, Collège de France, Paris, France*
†Institutions Universitaires de Psychiatrie-Genève, Chêne-Bourg, Switzerland
and ‡INSERM U 266, CNRS UA 498, 4 av. de l'Observatoire, Paris, France

Received 23 October 1990

DE SAINT HILAIRE, Z., B. P. ROQUES AND S. NICOLAÏDIS. *Effect of a highly selective central CCK-B receptor agonist: BC-264 on rat sleep.* PHARMACOL BIOCHEM BEHAV **38**(3) 545–548, 1991.—The possible involvement of the CCK-B receptor type in the atypical somnolence and EEG changes induced by low doses of CCK-B was investigated by intraperitoneal administration of three different doses (8, 16 and 32 μg/kg) of the new highly potent and selective CCK-B agonist, BC-264, on sleep parameters in the fasted rat. At the dose of 8 μg/kg BC-264 induced a significant increase in waking in the second 120 min of recording without effect on slow wave sleep (SWS). BC-264 did not modify the others sleep parameters. Taken all together these results suggest that CCK-B type receptors are probably not critically involved in satiety and sleep.

BC-264 CCK-B receptors Sleep Rat

IT has been reported that systemic administration of CCK-B inhibits food intake in many animal species and humans (1, 9, 10, 17). CCK-8 has also been frequently studied for its effects on the terminal behavioral elements of satiety, resting and sleep. However, studies on sleep following CCK-8 administration have provided contradictory results. CCK-8 has been reported to have no significant effect on sleep after either intracerebroventricular or IP administration (14,15). Other studies found that CCK-8 was indeed able to promote sleep when food was not available (12). Because CCK-8 exhibits the same affinity for the peripheral type (CCK-A) and the central type (CCK-B) receptors, the involvement of these two receptors in satiety and sleep remains unclear.

The CCK-B receptor was shown to be abundant in various rat brain regions, including the cerebral cortex, olfactory bulbs, hippocampus, amygdala, nucleus accumbens and nucleus tractus solitarius (8,13). In contrast, CCK-A binding sites are found to be restricted to few rat brain regions (area postrema, nucleus tractus solitarius, interpenduncular nucleus) (13).

We have recently shown that a low dose of CCK-8, although anorexigenic, induced atypical somnolence and EEG changes (5). In this study, the appearance of irritative EEG raised the question whether CCK-8 acts on brain structures or only at the peripheral level.

The present work was therefore undertaken to evaluate further the contribution of each type of receptor upon the sleep-waking cycle in the rat by using a new CCK-related peptide BC-264: Boc-Tyr(SO_3H)-gNle-mGly-Trp-(NMe)-Nle-Asp-Phe-NH_2. Based on biochemical, behavioral and electrophysiological studies, BC-264 has been characterized as a potent and highly specific agonist for central CCK-B receptor (3, 6, 7).

On the other hand, BC-264, which is highly resistant to peptidases (16), has been found in the brain 15 minutes after systemic administration of its tritiated analog [^{3}H]pBC-264, indicating that this new compound can be given by systemic routes in order to investigate the behavioural responses induced by selective stimulation of CCK-B binding sites (16).

Therefore, this compound could be used for in vivo pharmacological studies, including sleep. Moreover, its lack of interaction with peripheral A-type receptors will help distinguish between direct central effects and those of peripheral origin elicited at A-type receptors and secondarily to the brain by peripheral afferents.

METHOD

Twenty-four male Wistar rats, weighing 250–300 g (Iffa Credo), were used. Under pentobarbital (Nembutal, 40 mg/kg) anesthesia, rats were surgically implanted with cortical electrodes to allow chronic electroencephalographic (EEG) recording as described previously (4). Immediately after surgery, rats were housed in Plexiglas test chambers which accommodated a flexible EEG

[1]Requests for reprints should be addressed to Z. de Saint Hilaire, Neurobiologie des Régulations, Collège de France, 11 Place Marcelin Berthelot, 75005 Paris, France.

TABLE 1

EFFECTS OF BC-264 ON SLEEP

	18.00–20.00 h		20.00–22.00 h	
	Control	BC-264	Control	BC-264
W	65 ± 22	74 ± 13	61 ± 22	85 ± 9*
SWS	48 ± 17	41 ± 11	46 ± 16	30 ± 7.5
PS	7 ± 5	5 ± 4	13 ± 10	4.5 ± 4
PS Episodes (mean)	2.3 ± 1	1.4 ± 0.7	2.8 ± 2	2 ± 10

*$p<0.05$ (paired t-test experimental vs. control).

Total amount (in min) of sleep variables (expressed as mean ± S.E.M.) during the 4 h (18.00–22.00 h) under control NaCl and after BC-246 (8 μg/kg).

TABLE 2

EFFECTS OF BC-264 ON SLEEP

	18.00–20.00 h		20.00–22.00 h	
	Control	BC-264	Control	BC-264
W	65 ± 22	74 ± 26	61 ± 22	78 ± 32
SWS	48 ± 17	41 ± 26	46 ± 16	33 ± 25
PS	7 ± 5	5 ± 2	13 ± 10	9 ± 7
PS Episodes (mean)	2.3 ± 1	1.3 ± 5*	2.8 ± 2	1.5 ± 0.2

*$p<0.05$ (paired t-test experimental vs. control).

Total amount (in min) of sleep variables (expressed as mean ± S.E.M.) during the 4 h (18.00–22.00 h) under control NaCl and after BC-264 (16 μg/kg).

recording cable. The animals recovered from surgery for at least 5 days before electrodes were connected to the cables. During this period, standard lab chow (Extalabo M25) and water were always available. Cables were attached through electronic swivels (Air precision) to a polygraph (Beckman Accutrace). Room lights were on from 06.00 h to 18.00 h and ambient temperature was maintained at 24 ± 1°C. Food was removed 4 h before recordings, i.e., at 14.00 h. All recordings began at 18.00 h and lasted for 6 h. No food was available during the recording time. BC-264 was freshly dissolved in physiological saline (0.15 M NaCl) immediately before each experiment. At 18.00 h, each rat was removed from its cage, injected intraperitoneally with one dose of BC-264 or an equal volume of vehicle as a control, and returned to the test chamber. All rats received the following types of injections in a random fashion: saline, BC-264 8, 16 and 32 μg/kg.

According to a double-blind procedure, 3 different stages of sleep were visually counted for waking (W), slow wave sleep (SWS) and paradoxical sleep (PS). The visual scoring was performed on the first 4 h of recordings. For EEG patterns, the following criteria were adopted: Periods of SWS of less than 20 s within a waking period were not distinguished from waking. PS was identified only if the event lasted more than 10 s. The characteristic preceding transition period (5–10 s of high-voltage low-frequency waves modulating high-frequency regular waves) was not included in the duration of PS. Differences between groups were tested for statistical significance by using Student's t-test.

RESULTS

The effect of BC-264 (8, 16 and 32 μg/kg IP) on the rat sleep-wake cycle was examined. Tables 1, 2 and 3 show the effects of these doses on the duration of wakefulness, SWS, PS and PS episodes. Administration of BC-264 had no effect on the latency to the first episode of sleep. There was no effect on W, SWS and PS. However, at the dose of 8 μg/kg, BC-264 caused a slight increase in the duration of waking. The change in waking was significant in the second 120 min of recording. No significant difference was observed with the two high doses. Table 2 shows that the moderate dose (16 μg/kg) decreased the mean duration of PS. With the two other doses (8 and 32 μg/kg), the mean of PS episodes did not differ from controls.

Thus none of the sleep data studied, waking, SWS, and PS, were significantly different from those obtained after NaCl 0.15 M. In a separate pilot study, four additional rats were injected IP with 500 and 1000 μg/kg of BC-264. In neither animal was a change in sleep parameters seen. The behavior of all animals was observed during one hour postinjection and no discrepancies between behavior and EEG were noticed (such as immobility in the presence of an aroused EEG pattern).

All animals exhibited normal postures and motor coordination. At no time did the animals show any signs of distress following the injection that might have interfered with sleep. Again, BC-264 did not bring about any abnormal or even unusual EEG signs that could be indications of malaise (5) (Fig. 1).

This study was conducted under conditions that minimize endogenous factors that influence sleep. Since rats are active during the dark phase of the day-night cycle, the threshold for sleep should be high and endogenous factors that influence the onset and duration of sleep should be at their nadirs. In addition, food was not available, which eliminated the possible influence of endogenously released CCK, or any other peptides released or activated by ingestion which could complicate the analysis. The doses of BC-264 used here for systemic administration were similar to those used in our recent study with CCK-8 (5).

DISCUSSION

Our results indicate, therefore, that in contrast to CCK-8, BC-264 does not promote sleep in rats at doses of 8 to 32 μg/kg. Thus administration of BC-264 was not followed by a decrease of sleep latency or an increase in the duration of slow wave sleep. Furthermore, there was no change in the pattern of EEG (Fig. 1). Again, this result is in direct contrast with our recent findings (5), showing that immediately after similar doses of CCK-8 (8, 16

TABLE 3

EFFECTS OF BC-264 ON SLEEP

	18.00–20.00 h		20.00–22.00 h	
	Control	BC-264	Control	BC-264
W	65 ± 22	59 ± 15	61 ± 22	69 ± 14
SWS	48 ± 17	54 ± 17	46 ± 16	45 ± 15
PS	7 ± 5	7 ± 2	13 ± 10	6 ± 3
PS Episodes (mean)	2.3 ± 1	2.2 ± 1	2.8 ± 2	2 ± 0.2

Total amount (in min) of sleep variables (expressed as mean ± S.E.M.) during the 4 h (18.00–22.00 h) under control NaCl and after BC-264 (32 μg/kg).

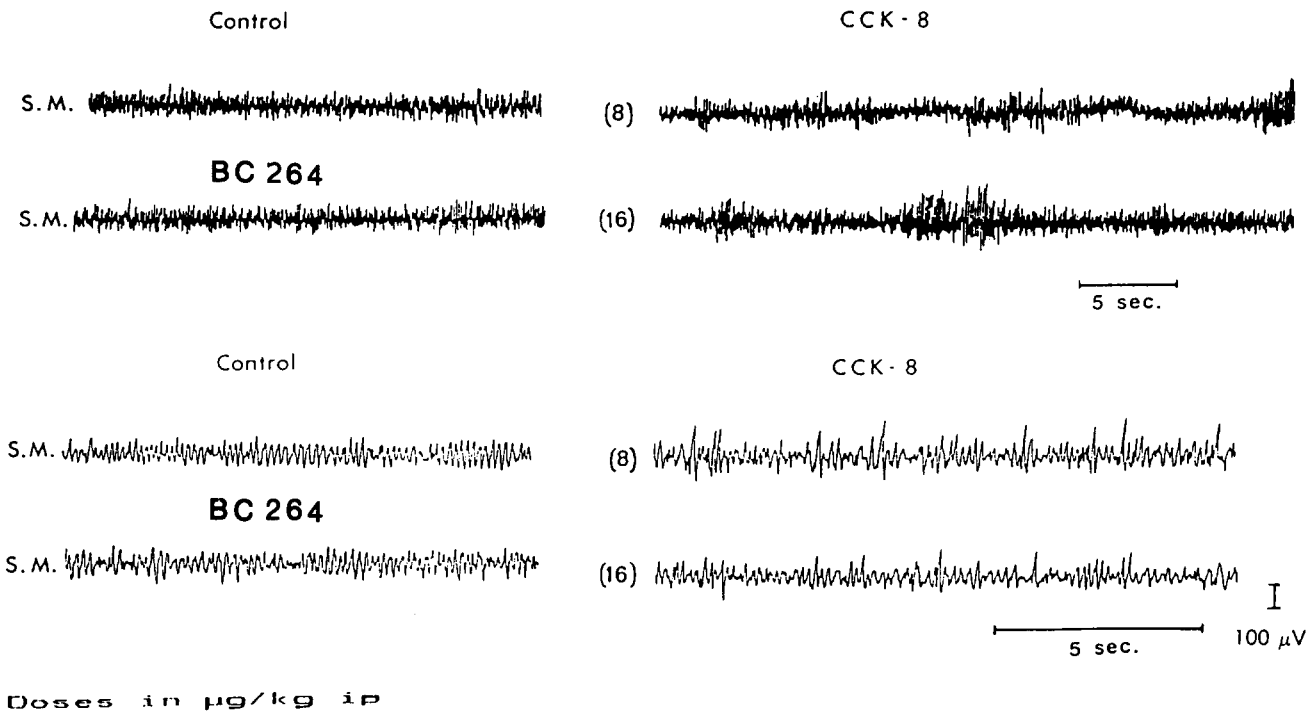

FIG. 1. Effect of BC-264 (8, 16 and 32 μg/kg) and CCK-8 (8 and 16 μg/kg) on sensorimotor cortex in comparison with control. Spindle wave episodes and isolated spikes are immediately elicited after CCK-8 administration.

and 32 μg/kg), unusual acute spikes were observed, during which the rats' eyes were open and the animals displayed awake immobility.

According to these findings, Crawley et al. (4) have recently shown that BC-264 administered by ICV (20 ng to 5 μg) or IP (5–50 μg/kg) routes in rats did not reduce food intake, whereas lower doses of CCK-8 do so (9), demonstrating that CCK-B type receptors are not involved in the anorexigenic action of the peptide.

In conclusion, the EEG and behavioral data taken collectively demonstrate that whatever the effect of CCK-8 on feeding and sleep, they are very likely exerted via peripheral (CCK-A) rather than central (CCK-B) receptor types. Therefore, the failure of BC-264, a highly potent and selective CCK-B receptor agonist to reduce food intake and to increase sleep, is of great interest in resolving the still controversial role of CCK in the regulation of satiety and sleep.

ACKNOWLEDGEMENTS

This work was supported by grants from INSERM and FRM. We thank B. Charpentier for the synthesis of BC-264 and C. Durieux for discussions.

REFERENCES

1. Antin, J.; Gibbs, J.; Holt, J.; Young, R. C.; and Smith, C. P. Cholecystokinin elicits the complete behavioral sequence of satiety in rats. J. Comp. Physiol. Psychol. 89:784–790; 1975.
2. Baile, C. A.; McLaughlin, C. L.; Della Ferra, M. A. Role of cholecystokinin and opioid peptides in control of food intake. Physiol. Rev. 66:172–234; 1986.
3. Charpentier, B.; Durieux, C.; Pélaprat, D.; Dor, A.; Reibaud, M.; Blanchard, J. C.; Roques, B. P. Enzyme-resistant CCK analogs with high affinities for central receptors. Peptides 9:835–842; 1988.
4. Crawley, J. N.; Fiske, S. M.; Durieux, C.; Derrien, M.; Roques, B. P. Centrally administered cholecystokinin suppresses feeding through a peripheral-type receptor mechanism. Submitted.
5. de Saint Hilaire, Z.; Depoortere, H.; Nicolaïdis, S. Does cholecystokinin induce physiological satiety and sleep? Brain Res. 488:304–310; 1989.
6. Daugé, V., Bohme, G. A., Crawley, J. N., Durieux, C., Stutzmann, J. M., Feger, J., Blanchard, J. C. and Roques, B. P. Investigation of behavioral and electrophysiological responses induced by selective stimulation of CCK-B receptors by using a new highly potent CCK analog: BC 264. Synapse; in press.
7. Durieux, C.; Corringer, P.-J.; Bergeron, F.; Roques, B. P. [^{3}H]pBC 264, first highly potent and very selective radioligand for CCK-B receptors. Eur. J. Pharmacol. 168:269–270; 1989.
8. Gaudreau, P.; St-Pierre, S.; Pert, C. B.; Quirion, R. Characterization and visualization of cholecystokinin receptors in rat brain using [^{3}H]pentagastrin. Peptides 4:755–762; 1983.
9. Gibbs, J.; Young, R. C.; Smith, G. P. Cholecystokinin decreases food intake in rats. J. Comp. Physiol. Psychol. 84:488–495; 1973.
10. Gibbs, J.; Falasco, J. D.; McHugh, P. R. Cholecystokinin decreases food intake in rhesus monkeys, Am. J. Physiol. 230:15–18; 1976.
11. Hill, D. R.; Campell, N. J.; Shaw, T. M.; Woodruff, G. N. Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J. Neurosci. 7:2967–2976; 1987.
12. Kapas, L.; Obal, F., Jr.; Alföldi, P.; Rubiseck, G.; Penke, B.; Obal, F. Effects of nocturnal intraperitoneal administration of cholecystokinin in rats: simultaneous increase in sleep, increase in EEG slow wave activity, reduction of motor activity, suppression of eating, and decrease in brain temperature. Brain Res. 438:155–164; 1988.
13. Pelaprat, D.; Broer, Y.; Studler, J. M.; Peschanski, M.; Tassin, J. P.; Glowinski, J.; Rostene, W.; Roques, B. P. Autoradiography of CCK receptors in the rat brain using [^{3}H] Boc [^{Nle} 28.31] CCK_{27-33} and [^{125}I] Boulton-Hunter CCK_8. Functional significance of subregional distributions. Neurochem. Int. 10:495–508; 1987.

14. Riou, F.; Cespuglio, R.; Jouvet, M. Endogenous peptides and sleep in the rat. II. Peptides without significant effect on the sleep-waking cycle. Neuropeptides 2:255–264; 1982.
15. Rojas-Ramirez, J. A.; Crawley, J. N.; Mendelson, W. B. Electroencephalographic analysis of the sleep-inducing actions of cholecystokinin. Neuropeptides 3:129–138; 1982.
16. Ruiz-Gayo, M.; Delay-Goyet, P.; Durieux, C.; Corringer, P. J.; Baamonde, A.; Gacel, G.; Roques, B. P. Investigation of opioid and CCK central receptors after peripheral injection of selective and enzyme-resistant peptides. J. Control. Reles.; in press.
17. Stacher, G. The effects of cholecystokinin and caerulein on human eating behavior and pain sensation. Psychoendocrinology 11:39–48; 1986.