Pharmacology Biochemistry and Behavior, Vol. 59, No. 1, pp. 261–264, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00416-4

Effect of a μ-Selective Opioid Antagonist on CCK-8–Induced Changes in Thermoregulation in the Rat

SUCHANDRA GHOSH,[1] CYNTHIA M. HANDLER, ELLEN B. GELLER[2] AND MARTIN W. ADLER

Department of Pharmacology, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140

Received 10 January 1997; Revised 1 May 1997; Accepted 14 May 1997

GHOSH, S., C. M. HANDLER, E. B. GELLER AND M. W. ADLER. *Effect of a μ-selective opioid antagonist on CCK-8-induced changes in thermoregulation in the rat.* PHARMACOL BIOCHEM BEHAV **59**(1) 261–264, 1998.—We examined the effects of ICV injection of CCK-8 alone and in combination with CTAP, a μ-selective antagonist, on brain surface temperature (Tb), oxygen consumption (VO_2), and heat exchange (Q) in unrestrained male S-D rats at an ambient temperature of 20 ± 0.5°C. CCK-8 (300 ng, ICV) produced hyperthermia (Δ Tb: 0.86 ± 0.22°C) lasting for 30–60 min, which was associated with an increase in VO_2 (1.42 ± 0.28 ml/g/h). There was an increase in Q (1.87 ± 1.2 cal/g/h) beginning 15 min after injection and lasting for 60 min. The CCK-8–induced hyperthermia was attenuated by a postinjection increase in Q. During the 60–120 min postinjection period, VO_2 and Q returned to baseline followed by the return of Tb to preinjection levels. CCK-8–induced increases in Tb, Q, and VO_2 were blocked by pretreatment with CTAP (0.75 nmol, ICV) 15 min earlier. CTAP alone did not significantly affect ΔTb, ΔVO_2, and ΔQ. These results suggest that, within the thermoneutral zone, the thermoregulatory effects of CCK-8 in the rat involve participation of μ-opioid receptors. ©1998 Elsevier Science Inc.

Mu opioid antagonist Cholecystokinin Thermoregulation Brain temperature Oxygen consumption

HOMEOTHERMS can maintain a constant body temperature (Tb) in spite of wide variations in environmental temperature by balancing heat production and heat loss. The regulation of Tb is achieved by a neural network, situated in the central nervous system (CNS), that exerts command functions over an array of control systems present throughout the body (3,4). Contributing to this network are neurons with opioid receptors that are found throughout the CNS and periphery. The exogenous administration of opioid agonists is known to produce changes in Tb that are dependent on a variety of factors such as species, strain, restraint, ambient temperature, dose, and route of drug administration (1,6). Cholecystokinin octapeptide (CCK-8), long recognized as an important gut peptide, has been shown to be widely distributed in the brain (2), and a possible role of CCK-8 in the modulation of body temperature regulation has been proposed (7). Intraperitoneal (IP) or subcutanous administration of CCK-8 produced hypothermia (15,17), while intracerebroventricular (ICV) injection caused hyperthermia (23,26).

Some evidence suggested that a physiological interaction between CCK-8 and the endogenous opioid system exists in the CNS (16). CCK-8 has been reported to act as an antagonist of opioids in analgesia, and a role of CCK-8 as an endogenous opioid antagonist has been postulated (8,12). However, Xin et al. (25) found that lower doses of CCK microinjected into the periaqueductal gray (PAG) antagonized, and higher doses enhanced, the analgesic effect of the μ opioid, PL-017. In addition, it has been suggested that CCK-8 may act as an endogenous agonist with opioids in the regulation of seizure susceptibility (18). Recently, we examined the CCK-opioid in-

[1]Current address: Clinical Research Center, R. W. J. Medical School, UMD-NJ, 1 Robert Wood Johnson Place, CN-19, New Brunswick, NJ 08903.

[2]Requests for reprints should be addressd to Ellen B. Geller, Department of Pharmacology, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140.

teraction in thermoregulation (11) by measuring rectal body temperature.

The aim of this study was to examine CCK/opioid interactions on the physiological mechanisms, heat-exchange (Q) and oxygen consumption (VO_2), which are responsible for the maintenance of Tb. To accomplish this goal, we used a gradient-layer calorimeter to measure Tb, VO_2, and Q simultaneously following administration of CCK-8 alone and in combination with a μ opioid antagonist, cyclic D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP), in the unrestrained rat.

METHOD

Male Sprague–Dawley rats (Zivic-Miller, Pittsburgh, PA), weighing approximately 200 g, were housed four to five per cage in an animal room maintained at 22 ± 2°C and 50 ± 10% relative humidity and on a 12 L:12 D cycle. Food and water were available ad lib. One week before testing, a polyethylene cannula was implanted into the right lateral ventricle of each rat under ketamine (150 mg/kg, IP) anesthesia. At the same time, a YSI thermistor (Yellow Springs Instrument, Yellow Springs, OH; resistance 2252 Ω at 25°C) was placed on the surface of the cortex over the left lateral ventricle. Cannula and thermistor were secured to the cranium with stainless steel machine screws and dental acrylic. Animals were housed individually after surgery and weighed 250 to 300 g at time of testing.

A gradient-layer calorimeter (inside dimensions, 21 × 14 × 10 cm) was flushed with air (20% O_2) and brought to testing temperature (20 ± 0.5°C) before the animal was placed in it. The rat was placed into the calorimeter and connected to the temperature cable and sealed into the chamber. The animal is not restrained and can move within the enclosure. Compressed air was fed into the chamber through a tube at the rate of 750 ml/min and was circulated by means of a small fan. VO_2, Q, and Tb [output measured in volts (9)] were recorded using MacLab™ and a MacClassic™ computer and were stored on diskette. Data were continuously recorded for 180 min before injection with the last 60 min serving as a baseline control for comparison with continuous recordings 180 min postinjection.

CCK-8 (RBI, Natick, MA) and CTAP (Peninsula Labs, Belmont, CA) were dissolved in pyrogen-free saline and were injected in a volume of 3 μl followed by a 3-μl saline flush. CTAP was administered 15 min prior to the injection of CCK-8. Doses of drugs were selected from full dose–response curves (11) for each drug using rectal body temperature at ambient temperature of 20°C as an end point. Drug solution was injected through the polyethylene cannula with the help of a Hamilton glass syringe without removing the animal from the calorimeter.

Results are reported as ΔTb (°C), ΔVO_2 (ml O_2/g body wt/h), and ΔQ (cal /g body wt/h) from baseline controls for each time interval. All results were expressed as mean ± SEM. Statistical analysis of difference between groups was assessed with a two-way analysis of variance (ANOVA) followed by a posthoc Fisher's test. A value of $p < 0.05$ was taken as the significant level of difference.

RESULTS

ICV administration of CCK-8 (300 ng, 0.25 nmol) significantly increased Tb ($p < 0.05$, compared to control by ANOVA and Fisher's test), with a peak at 60 min (0.86 ± 0.22°C; $p < 0.05$; $df = 11$), the onset occurring within 30 min after injection (0.65 ± 0.16 C) (Fig. 1). Tb did not differ significantly from basal value (35.41 ± 0.24°C) for the remainder of the postinjection period. ICV administration of CTAP (0.75 nmol) alone did not significantly change Tb. However, pretreatment with CTAP significantly reduced ($p < 0.05$ by ANOVA and Fisher's test) the CCK-8-induced increase in Tb (Fig. 1).

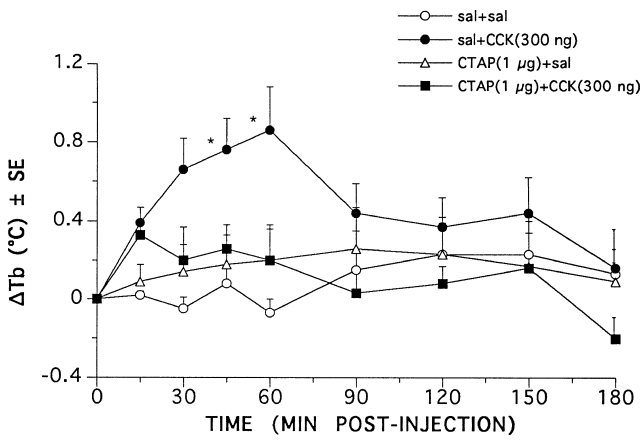

FIG. 1. Effect of CCK-8 (300 ng, ICV) and/or CTAP (1 g, ICV) on the change in brain surface temperature (Tb) at 20°C. Each point represents the mean of 5–10 animals ± SE. *$p < 0.05$.

Treatment with CCK-8 (300 ng) also increased VO_2 ($p < 0.05$, compared to control by ANOVA and Fisher's test), which lasted for 30–60 min following injection (Fig. 2). The increase in VO_2 reached a peak (1.42 ± 0.28 ml/g/h; $p < 0.05$; $df = 12$) 30 min after injection, lasted for 60 min (0.99 ± 0.27 ml/g/h), and returned to baseline values by 90 min. CTAP (0.75 nmol), which had no effect when injected ICV alone, blocked the CCK-8–induced rise in VO_2 (Fig. 2).

As shown in Fig. 3, CCK-8 produced a significant rise in Q (1.87 ± 1.2 cal/g/h; $p < 0.05$; $df = 12$, compared to control by ANOVA and Fisher's test) 15 min after injection. A maximum increase (2.77 ± 1.07 cal/g/h; $p < 0.05$; $df = 15$) was observed 30 min postinjection and lasted for 45–60 min (1.36 ± 0.73 cal/g/h). No significant change in Q was observed 90–180 min after injection. The CCK-8–induced increase in Q (Fig. 3) was partially attenuated by pretreatment with CTAP.

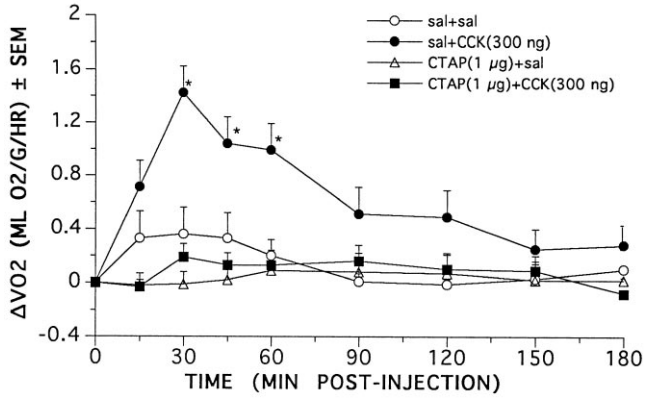

FIG. 2. Effect of CCK-8 (300 ng, ICV) and/or CTAP (1 g, ICV) on the change in oxygen consumption (VO_2) at 20°C. Each point represents the mean of 5–10 animals ± SE. *$p < 0.05$.

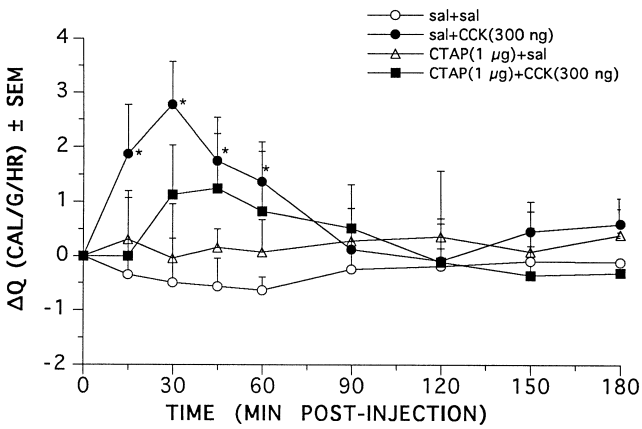

FIG. 3. Effect of CCK-8 (300 ng, ICV) and/or CTAP (1 g, ICV) on the change in heat exchange (Q) at 20°C. Each point represents the mean of 5–10 animals ± SE. *$p < 0.05$.

DISCUSSION

In mammals, a relatively constant body temperature is maintained by the balance between heat production and heat loss. Administration of neuropeptides has been shown to change Tb, depending on species, dosage, route of administration, and ambient temperature (5). The present study demonstrated that CCK-8 can modulate the physiological mechanisms that contribute to the maintenance of Tb. ICV injection of CCK-8 produced a hyperthermia that lasted for 30–60 min after injection. Increased VO_2 peaked 30 min after CCK-8 injection and plateaued for 30 min before returning to baseline. Similarly, Q peaked at 30 min postinjection, returning to baseline 60–90 min postinjection. Although VO_2 and Q increased simultaneously during the first 30 min postinjection, the hyperthermia was due to the longer duration of the increase in VO_2 compared to Q. Thus, Q/VO_2 decreased during the first hour post injection (2.63 at 15 min to 1.37 at 60 min), indicating that the increase in oxygen consumption was the major factor contributing to the CCK-induced rise in Tb. The increase in Q early in the postinjection period is not usually associated with hyperthermia. Typically, this pattern of heat loss is found later in the postinjection period and is believed to be at least partially responsible for reducing the increase in Tb (14). Early increases in heat loss have been associated with the induction of hypothermia (13). By 90 min postinjection, both VO_2 and Q have returned to control levels, with Tb following behind.

Changes in VO_2 reflect the amount of heat generated by the peripheral oxidative metabolism while Q represents the conservation or dissipation of that heat. During the first 15–30 min after CCK-8 administration, heat production increased, raising the quantity of available metabolic heat. Simultaneously, a compensatory mechanism was activated, resulting in peripheral vasodilation. It may be speculated that the heat generated by increases in oxidative metabolism could not be directed towards increasing Tb because of the activation of heat loss mechanisms, and that these events prevented a greater increase in Tb. After 30 min, heat loss (i.e., Q) decreased to baseline level more rapidly than heat production (i.e., VO_2). This represents a partial conservation of the generated heat, resulting in a small hyperthermia (ΔTb: 0.86°C) 45–60 min postinjection. After 60 min postinjection, Tb began to decline to baseline level as metabolic heat production and heat loss returned to baseline. Shian and Lin (21) have reported that direct administration of CCK-8 (20–60 ng) into the preoptic anterior hypothalamus (POAH) produced hypothermia in a cool environment (8–22°C) accompanied by a decrease in metabolism and an increase in cutaneous temperature (indicative of vasodilation). Szelényi et al. (23) have shown that, in the normothermic female rat exposed to an ambient temperature of 28–30°C, an ICV injection of CCK-8 in the dose range of 50–500 ng induced a thermogenic response with tail skin vasoconstriction and a resulting rise in colonic temperature. The differences in experimental conditions such as sex, ambient temperature, mode of injection, dose, and lack of direct measurement of Q may account for the discrepancy in the literature regarding the changes in oxidative metabolism and heat exchange. A hypothermic effect of peripherally administered CCK-8 in the rat (17) could result from a direct skin vasodilation caused by the peptide without a coordinated CNS effect on temperature regulation.

In a recent study using rectal temperature as the end point, we have found that ICV administration of CCK-8 regulated body temperature through interaction with the endogenous μ-opioid system (11). In the present study, the μ-selective antagonist, CTAP, was used to probe the interaction between CCK-8 and the opioid system in terms of the modulation of thermoregulatory mechanisms. The role of CTAP as a μ-selective antagonist in thermoregulation has been demonstrated (14). Pretreatment with CTAP (0.75 nmol) completely blocked the changes in Tb and VO_2 produced by PL-017 (1.86 nmol), a μ-selective agonist. Our results showed that a 15-min pretreatment with CTAP blocked the CCK-induced rise in VO_2, which resulted in the blockade of CCK-induced hyperthermia. These results suggest that CCK-8 altered peripheral thermoregulatory mechanisms through interaction with the endogenous μ opioid system. Morphine, which primarily acts at μ-opioid receptors, has been reported to produce hyperthermia at lower doses (e.g., 4 mg/kg, SC), associated with cutaneous vasoconstriction, increased metabolic heat production, and oxygen consumption (27). A high dose (e.g., 64 mg/kg, SC) of morphine, on the other hand, causes hypothermia generally associated with cutaneous vasodilation and decreased metabolic heat production. Changes in VO_2 and Q may be induced by μ-selective agonists acting on central receptors. Spencer et al. (22) have reported that ICV injection of DAMGO, another μ-selective agonist, caused hyperthermia, which was facilitated by the selection of a warm ambient temperature. Handler et al. (14) have shown that within the thermoneutral zone, μ-receptor stimulation by the highly selective agonist, PL-017 (1.86 nmol) produces hyperthermia as a result of an immediate increase in VO_2 and a concurrent reduction in Q. Return of Tb to baseline was the result of an increase in heat loss and followed a return of the metabolic heat production to baseline. In the present study, an increase in Tb following CCK administration was the result of an increase in metabolic heat generation and a partial conservation of that heat. Simultaneous activation of heat loss mechanisms attenuated the CCK-8–induced hyperthermia. Thus binding of endogenous CCK-8 at CCK receptors increased the activity of endogenous opioid receptors, suggesting that the opioid system may be involved in thermic responses to CCK-8.

The thermoregulatory effect of CCK-8 may be correlated to the distribution of this peptide in the CNS. The exact neuroanatomical location of the CCK–opioid interaction is unknown. CCK-8 has been shown to be localized most abundantly in the POAH, which is important in the regulation of

Tb (19). The POAH has been established as the primary site of morphine thermoregulatory effects (5). In rats, ICV morphine produces a primarily hyperthermic effect (10), probably by affecting a central control system. Szelényi et al. (23) have suggested that the thermogenic response of CCK-8 is mediated by central CCK_B receptors. CCK_B receptors have also been found to be involved in the enhanced analgesic effect of the agonist PL-017 when CCK (20–120 ng) is microinjected into PAG (25). CCK_B receptors may participate in the pathogenesis of lipopolysaccharide-induced fever in rats (24). In guinea pigs, the opioid receptor antagonist naloxone has been shown to have a peripheral antipyretic action, suggesting that circulating opioids also may have a role in fever production (20).

In summary, our results demonstrate that ICV administration of CCK-8 increased oxidative metabolism. Heat exchange mechanisms were also activated, reducing Tb through the dissipation of heat generated through the increase in metabolic rate. The selective μ receptor antagonist, CTAP, blocked these alterations in metabolic rate and partially attenuated the increase in heat exchange. These results suggest that CCK-8 may produce its effects on Tb and thermoregulatory mechanisms through the endogenous μ-opioid system.

ACKNOWLEDGEMENTS

This work was supported by an INVEST Fellowship from NIDA to S. G. and Grant DA 00376 from NIDA.

REFERENCES

1. Adler, M. W.; Geller, E. B.; Rosow, C. E.; Cochin, J.: The opioid system and temperature regulation. Annu. Rev. Pharmacol. Toxicol. 28:429–449; 1988.
2. Beinfeld, M. C.: Cholecystokinin in the central nervous system: A minireview. Neuropeptides 3:411–427; 1983.
3. Blatteis, C. M.: Hypothalamic substances in the control of body temperature: General characteristics. Fed. Proc. 40:2735–2740; 1981.
4. Bligh, J.: The central neurology of mammalian thermoregulation. Neuroscience 4:1213–1235; 1979.
5. Burks, T. F.: Opioids and opioid receptors in thermoregulation. In: Schönbaum, E.; Lomax, P., eds. Thermoregulation: Pathology, pharmacology and therapy. New York: Pergamon; 1991:489–508.
6. Clark, W. G.: Effects of opioid peptides on thermoregulation. Fed. Proc. 40:2754–2759; 1981.
7. Clark, W. G.; Lipton, J. M.: Changes in body temperature after administration of amino acids, peptides, dopamine, neuroleptics and related agents: II. Neurosci. Biobehav. Rev 9:299–371; 1985.
8. Faris, P. L.; Komisaruk, B. R.; Watkins, L. R.; Mayer, D. J.: Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. Science 219:310–312; 1983.
9. Geller, E. B.; Adler, M. W.: Drugs of abuse and body temperature. In: Adler, M. W.; Cowan, A., eds. Testing and evaluation of drugs of abuse. New York: Wiley-Liss; 1990:101–119.
10. Geller, E. B.; Rowan, C. H.; Adler, M. W.: Body temperature effects of opioids in rats: Intracerebroventricular administration. Pharmacol. Biochem. Behav. 24:1761–1765; 1986.
11. Ghosh, S.; Geller, E. B.; Adler, M. W.: Interaction of cholecystokinin and somatostatin with a selective mu opioid agonist and mu and kappa antagonists in thermoregulation. Brain Res. 745:152–157; 1997.
12. Han, J.-S.; Ding, X.-Z.; Fan, S.-G.: Is cholecystokinin octapeptide (CCK-8) a candidate for endogenous anti-opioid substrates? Neuropeptides 5:399–402; 1985.
13. Handler, C. M.; Bradley, E. A.; Geller, E. B.; Adler, M. W.: A study of the physiological mechanisms contributing to neurotensin-induced hypothermia. Life Sci. 54:95–100; 1993.
14. Handler, C. M.; Geller, E. B.; Adler, M. W. Effect of μ-, κ-, and δ-selective opioid agonists on thermoregulation in the rat. Pharmacol. Biochem. Behav. 43:1209–1216; 1992.
15. Kandasamy, S. B.; Williams, B. A.: Cholecystokinin-octapeptide-induced hyperthermia in guinea-pigs. Experientia 39:1282–1284; 1983.
16. Kapás, L.; Benedek, G.; Penke, B.: Cholecystokinin interferes with the thermoregulatory effect of exogenous and endogenous opioids. Neuropeptides 14:85–92; 1989.
17. Kapás, L.; Obál, F., Jr.; Penke, B.; Obál, F.: Cholecystokinin-octapeptide-induced hypothermia in rats: Dose–effect and structure–effect relationships, effect of ambient temperature, pharmacological interactions and tolerance. Neuropharmacology 26:131–137; 1987.
18. Legido, A.; Adler, M. W.; Karkanias, C.; Geller, E. B.; Bradley, E.; Greenstein, J. I.; Grover, W. D.: Cholecystokinin potentiates morphine anticonvulsant action through both CCK-A and CCK-B receptors. Neuropeptides 28:107–113; 1995.
19. Miceli, M. O.; van der Kooy, D.; Post, C. A.; Della-Fera, M. A.; Baile, C. A.: Differential distributions of cholecystokinin in hamster and rat forebrain. Brain Res. 402:318–330; 1987.
20. Romanovsky, A. A.; Shido, O.; Ungar, A. L.; Blatteis, C. M.: Peripheral naloxone attenuates lipopolysaccharide fever in guinea pigs by an action outside the blood–brain barrier. Am. J. Physiol. 266:R1824–31; 1994.
21. Shian, L. R.; Lin, M. T.: Effects of cholecystokinin octapeptide on thermoregulatory responses and hypothalamic neuronal activity in the rat. Naunyn Schmiedebergs Arch. Pharmacol. 328:363–367; 1985.
22. Spencer, R. L.; Hruby, V. J.; Burks, T. F.: Alteration of thermoregulatory set point with opioid agonists. J. Pharmacol. Exp. Ther. 252:696–705; 1990.
23. Szelényi, Z.; Barthó, L.; Székely, M.; Romanovsky, A. A.: Cholecystokinin octapeptide (CCK-8) injected into a cerebral ventricle induces a fever-like thermoregulatory response mediated by type B CCK-receptors in the rat. Brain Res. 638:69–77; 1994.
24. Székely, M.; Szelényi, Z.; Balasko, M.: Cholecystokinin participates in the mediation of fever. Pflugers Arch. Eur. J. Physiol. 428:671–673; 1994.
25. Xin, L.; Geller, E. B.; Sterling, G. H.; McCafferty, M. R.; Adler, M. W.: Interactions between cholecystokinin (CCK) and selective μ and κ opioid receptor agonists in the periaqueductal gray (PAG) of the rat. NIDA Res. Monogr. 153:117; 1995.
26. Zetler, G.: Cholecystokinin octapeptide, caerulein and caerulein analogues: Effects on thermoregulation in the mouse. Neuropharmacology 21:795–801; 1982.
27. Zwil, A. S.; Lynch, T. J.; Martinez, R. P.; Geller, E. B.; Adler, M. W.: Calorimetric analysis of ICV morphine in the rat. In: Harris, L. S., ed. Problems of drug dependence 1987, NIDA Research Monograph #81. Washington, DC: U.S. Government Printing Office; 1988:285.