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The Effect of Caffeine on Food Intake in Rats: Involvement of Corticotropin-Releasing Factor and the Sympatho-Adrenal System

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RACOTTA, I. S., J. LEBLANC AND D. RICHARD. *The effect of caffeine on food intake in rats: Involvement of corticotropin-releasing factor and the sympatho-adrenal system.* PHARMACOL BIOCHEM BEHAV **48**(4) 887–892, 1994. – The involvement of CRH and the sympatho-adrenal system in the effects of caffeine on food intake and body weight gain has been investigated in rats. Food intake and body weight gain were measured in male rats after the treatment with caffeine in combination with either an injection of the CRH antagonist α-helical $CRH_{(9-41)}$, a surgical adrenal demedullation (medullectomy), or a ganglionic blockade. Alpha-helical $CRH_{(9-41)}$ was injected in the lateral ventricle of the brain and hexamethonium was used to chemically block the ganglionic transmission. From 4 to 24 h following a caffeine injection, spontaneous food intake, which was cumulated from the time caffeine was injected, was significantly ($p < 0.01$) lower in caffeine- than in saline-treated rats. In food-deprived rats, the anorectic effect of caffeine was biphasic, being significant at 0.5 and 1 h after the caffeine administration, then vanishing for 3 h, and becoming significant again 6 h after the caffeine administration. In both the spontaneously fed and food-deprived rats, caffeine reduced the rate of weight gain, which was measured at the end of a 12- or a 24-h period following the caffeine injection. A significant ($p < 0.05$) interaction effect of caffeine and α-helical-$CRH_{(9-41)}$ was found on the cumulative food intake at 1, 6, and 8 h, and on the amount of food eaten between the 4–6-h interval following the injection of caffeine; the effects of caffeine on food intake and body weight gain seem largely prevented by the use of a CRH antagonist. Neither the medullectomy nor the ganglionic blockade attenuated the effect of caffeine on food intake. There was, in fact, no interaction effect of either caffeine and medullectomy or caffeine and hexamethonium on postcaffeine food intake and body weight gain. The present results do not support a role for the sympatho-adrenal system in the effects of caffeine on food intake and body weight. This study, together with providing evidence for a central CRH-mediated anorectic action of caffeine, further emphasizes the role of CRH in the control of food intake.

Alpha-helical $CRH_{(9-41)}$ Adrenaline Adrenal demedullation Hexamethonium Ganglionic blockade

THE effects of caffeine on food intake have only scantily been addressed but there is indication that caffeine may suppress food intake. Indirect evidence supporting an anorectic role of caffeine arose from the suggestion that 75% of the weight loss following administration of caffeine and ephedrine was due to the effect of such a mixture on food intake (1,2). More direct support for the anorectic effect of caffeine had been provided by Tremblay and co-workers (23), who observed an acute reduction of 22% in spontaneous feeding in men treated with caffeine. Finally, caffeine had also been reported to produce symptoms of anorexia in patients with anxiety disorders (5).

The mechanisms governing the potential anorectic effects of caffeine have not been investigated and, in that respect, the present study was designed to assess the potential roles of corticotropin-releasing factor (CRH) and of the sympatho-adrenal system in the anorectic effect of caffeine on food intake. CRH (8,16) and peripheral catecholamines (20,21) are significant anorectic agents, whose secretions are likely enhanced by caffeine (3,10,13,15,17). In addition, the hypothesis that caffeine might affect food intake via successively stimulating CRH neural activity and adrenal catecholamines secretion warranted verification prior to designing this study,

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as CRH has been claimed to act through the sympatho-adrenal system to decrease food intake (4,9). In a first series of experiments, the effects of caffeine on food intake were characterized in rats. In a second series, the potential involvement of CRH was evaluated by treating rats with the CRH antagonist α-helical-CRH$_{(9-41)}$ prior to an injection of caffeine. In a third series, the implication of the sympatho-adrenal system in the anorectic effects of caffeine was assessed by testing the effects of caffeine in rats after adrenal demedullation (medullectomy) and ganglionic blockade. The latter series of experiments was based on the observation that caffeine by stimulating the sympathoadrenal system may suppress food intake through some peripheral metabolic actions of adrenaline and noradrenaline (20,21).

METHOD

Animals and Food Intake Measurements

Male Wistar rats weighing approximately 300 g were purchased from the Canadian Breeding Laboratories (St-Constant, Canada). All rats were cared and handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals, which is approved by the Natural Sciences and Engineering Research Council of Canada. Upon their arrival in our laboratory, the rats were housed singly in wire-bottom cages suspended above absorbent paper. They were maintained under controlled temperature (23 ± 1°C), on a 12L : 12D cycle, the lights being turned off between 0900 and 2100 h. Tap water and stock diet (Purina Chow # 5001) were available ad lib.

Experimental Procedures

Food intake measurements. In each of the experiments described below, food intake was measured at various time points following a caffeine injection. More specifically, food intake measurements were carried out at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 12.0, and 24.0 h after the caffeine administration, which occurred 15 min prior the onset of the dark period. Except for the first series of experiments, in which food intake measurements were performed in ad lib fed (spontaneously fed), food intake measurements were done in rats deprived of food for 12 h before the injection of caffeine. Caffeine (Research Biochemicals Incorporated, Natick, MA) was dissolved in sterile saline and injected intraperitoneally (IP) at a dose of 50 mg/kg (17) in a volume of 2.5 ml/kg. Depending on the series of experiments carried out, numbers of 8 to 12 rats were used in each experiment. Food intake measurements were carried out as follows. Feeders filled with powdered stock diet, were weighed, temporarily fixed inside cages, and removed and weighed when required. When perceptible, food spilled on the absorbent paper, above which the cages were suspended, was collected and accounted for. To minimize food spillage, a wire-mesh, which did not prevent rats from normally eating, was set up inside the feeder, on the top of the powdered stock diet. At the time each series of experiment began, rats had been accustomed, for at least a week, to the feeders, to the powdered diet, to the frequent removal of the feeders and to being weighed. During the dark phase of the cycle, food intake determinations were carried out under partial darkness. The room door was left slightly opened to allow a minimum of light for the determination.

Spontaneous feeding vs. food deprivation. In a first series of experiments, the effects of caffeine on food intake was assessed in spontaneously fed and food-deprived groups of rats. Following the caffeine injection, food intake measurements were carried out over a 24-h period at the time points enumerated above.

Caffeine and α-Helical-CRH$_{(9-41)}$. In this series of experiments, ten rats were first anesthetized (IP injection) with 3 ml/kg of a mixture of ketamine (20 mg/ml) and xylazine (2.5 mg/ml) and then stereotaxically implanted with a permanent guide cannula (Plastics One, Roanoke, VA) aimed at the right lateral ventricle of the brain. Following a 2-week recovery period from surgery, each rat received, at 3-day intervals, the four following treatments: intracerebroventricular (ICV) injection of saline with IP injection of saline, ICV injection of saline with IP injection of caffeine, ICV injection of α-helical-CRH$_{(9-41)}$ with IP injection of saline, and ICV injection of α-helical-CRH$_{(9-41)}$ with IP injection of caffeine. All rats did not receive the treatments in the same order. Food intake was measured in food-deprived rats as described above.

Alpha-helical-CRH$_{(9-41)}$ (1 mg) was added to 185 μl of sterile saline to which was added 15 μl of NaOH (1 N) to render the compound soluble. It was injected at a dose of 25 μg/rat in a volume of 5 μl 15 min prior to the IP injection of caffeine or saline. Alpha-helical-CRH$_{(9-41)}$ was kindly provided by J. Rivier from the Salk Institute, LaJolla, CA. Two-way repeated measures ANOVA was used to determine the main and interaction effects of caffeine and α-helical-CRH$_{(9-41)}$ on food intake and body weight measurements (11).

Caffeine and medullectomy. In this series of experiments, 24 rats were either bilaterally adrenal medullectomized (n = 12) or sham operated (n = 12). The medullectomies were surgically achieved under halothane anesthesia. Following a 7-day period of recovery, all animals from both medullectomized and sham-operated groups of rats were subjected, in a different order, to both the caffeine and the saline injection. Viability of the medullectomy was verified by the determination of the catecholamine (CA) contents of the adrenal gland at the end of the experiment. Tissue CA contents were assessed by HPLC with electrochemical detection (18). Rats with an adrenal CA content exceeding 5% of the total content found in sham-operated rats were discarded; on this basis, 4 out of 12 medullectomized rats were eliminated. Two-way ANOVA for repeated and independent measures were used to determine the main and interaction effect of caffeine (repeated measure) and medullectomy (independent measure) on food intake and body weight measurements (11).

Caffeine and ganglionic blockade. In this series of experiments, the effects of caffeine were assessed in a group of 12 rats treated with either 15 mg/kg (4) of hexamethonium (Research Biochemicals Incorporated, Natick, MA) or saline prior to the injection of caffeine. Hexamethonium was dissolved in sterile saline in a volume of 1 ml/kg. The injections took place 90 min before the IP injection of caffeine or saline. Each rat received the four following treatments at 3-day intervals: IP injection of saline with IP injection of saline, IP injection of saline with IP injection of caffeine, IP injection of hexamethonium with IP injection of saline, and IP injection of hexamethonium with IP injection of caffeine. All rats did not receive the treatments in the same order. Food intake was measured in food-deprived rats as described above. Two-way repeated measures ANOVA was used to determine the main and interaction effect of caffeine and hexamethonium on food intake and body weight measurements (11).

RESULTS

Spontaneous Feeding vs. Food Deprivation

From 4 to 24 h following the caffeine injection, spontaneous food intake, which was cumulated from the time of caffeine injection, was significantly ($p < 0.01$) lower in caffeine- than in saline-treated rats (Fig. 1, panel A). The body weight gain of the spontaneously fed rats, which was measured at the end of the 12- and 24-h periods following the caffeine injection (Fig. 1, panel C), was also lower in caffeine- than in saline-treated rats ($p < 0.05$). In food-deprived rats, the lowering effect ($p < 0.05$) of caffeine on food intake was apparent at 0.5 and 1 h after the caffeine administration (Fig. 1, panel B), then vanished for 3 h, and became significant again 6 h from the time caffeine was administered. Body weight gain of food-deprived rats was significantly ($p < 0.05$) lower in caffeine- than in saline-injected rats at the end of the 12- and 24-h periods following the caffeine injection (Fig. 1, panel D).

Caffeine and α-Helical-CRH$_{(9-41)}$

Figure 2 illustrates the respective and interactive influence of α-helical-CRH$_{(9-41)}$ and caffeine on food intake and body weight gain in food-deprived rats. A main effect ($p < 0.05$) of caffeine on cumulative food intake was observed at 0.5 and 24 h following the caffeine injection (panel A). A similar effect of caffeine ($p < 0.05$) was observed on food intake dur-

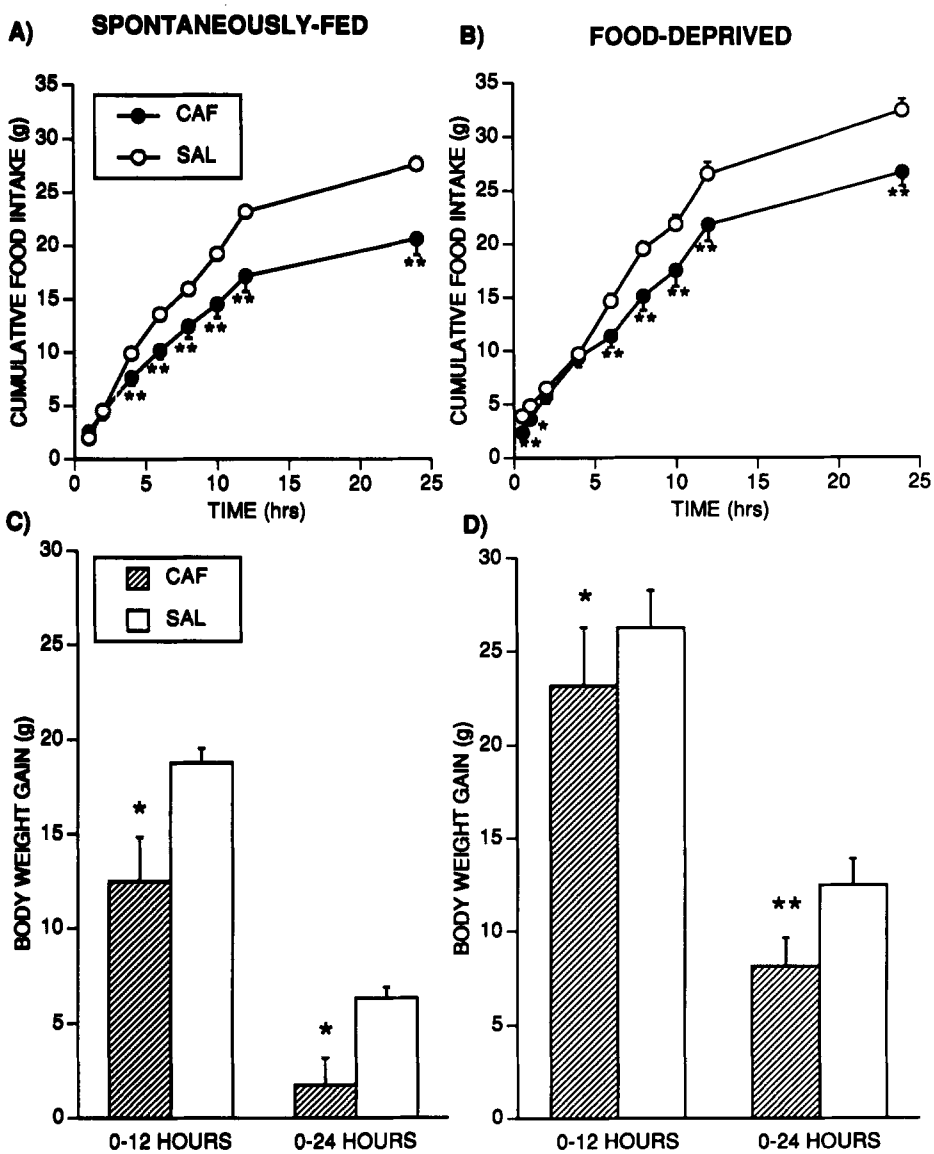

FIG. 1. Effect of caffeine on food intake (panel A and B) and body weight gain (panel C and D) in spontaneously fed (panel A and C) and food-deprived rats (panel B and D) (see the Method and Results sections for further details). Asterisks indicate the presence of significant differences between groups at the probability levels of 95 (*) or 99 (**) percent.

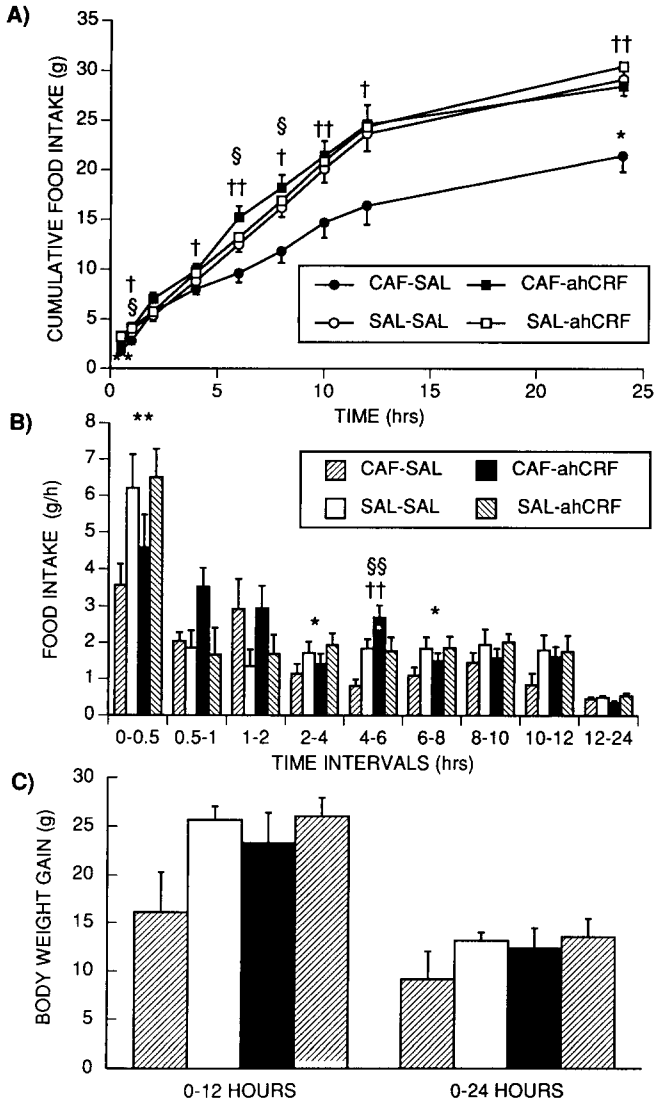

FIG. 2. Respective and interactive effects of caffeine and α-helical $CRH_{(9-41)}$ on cumulated (panel A) and noncumulated (panel B) food intake, and on body weight gain (panel C) in food-deprived rats (see the Method and Results sections for further details). Asterisks and daggers reveal significant main effects of caffeine and α-helical $CRH_{(9-41)}$, respectively, at the probability levels of 95 (*†) or 99 (**††) percent. The reference mark § reveal significant caffeine-α-helical $CRH_{(9-41)}$ interactions at the probability levels of 95 (§) or 99 (§§) percent.

ing the time intervals 0–0.5, 2–4, and 6–8 h following the injection of caffeine (panel B). A main effect ($p < 0.05$) of the α-helical-$CRH_{(9-41)}$ was observed on the cumulative food intake at 1, 4, 6, 8, 10, 12, and 24 h (panel A) and on the 4–6-h interval (panel B) following the caffeine injection. A significant ($p < 0.05$) interaction effect of caffeine and α-helical-$CRH_{(9-41)}$ was found on the cumulative food intake at 1, 6, and 8 h (panel A) and at the interval 4–6 h (panel B). In this series of experiments, caffeine and α-helical-$CRH_{(9-41)}$ did not exert any significant effect on the body weight gain during the 12- and 24-h periods following the caffeine injection (panel C).

Caffeine and Medullectomy

As shown in Fig. 3, there was no interaction effect of caffeine and medullectomy on food intake and body weight gain. The results expressed on Fig. 3 confirmed the anorectic and body weight-lowering effects of caffeine in food-deprived rats. In fact, caffeine reduced food intake during various time intervals following the caffeine injection (panel B) and blunted the body weight gain during the 12- and 24-h periods following the caffeine injection (panel C). Four hours following caffeine injection, medullectomized animals had a reduced cumulative food intake (panel A), which was accounted for by a smaller food intake between 2 and 4 h after the caffeine injection

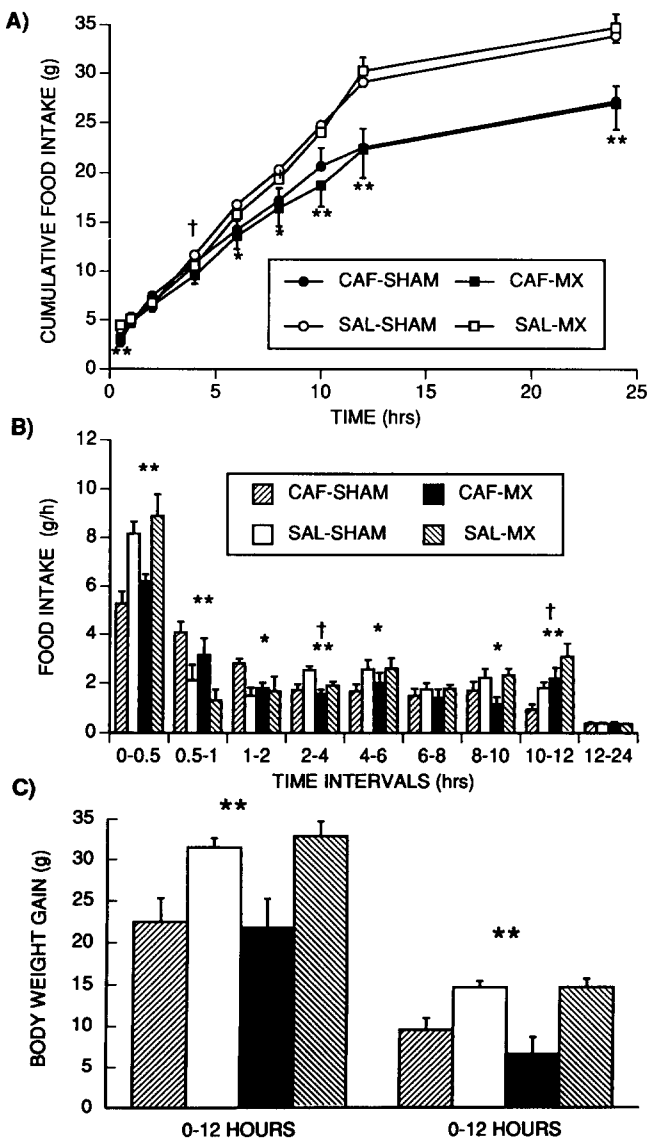

FIG. 3. Respective and interactive effects of caffeine and medullectomy on cumulated (panel A) and noncumulated (panel B) food intake, and on body weight gain (panel C) in food-deprived rats (see the Method and Results sections for further details). Asterisks and daggers reveal significant main effects of caffeine and medullectomy, respectively, at the probability levels of 95 (*†) or 99 (**††) percent.

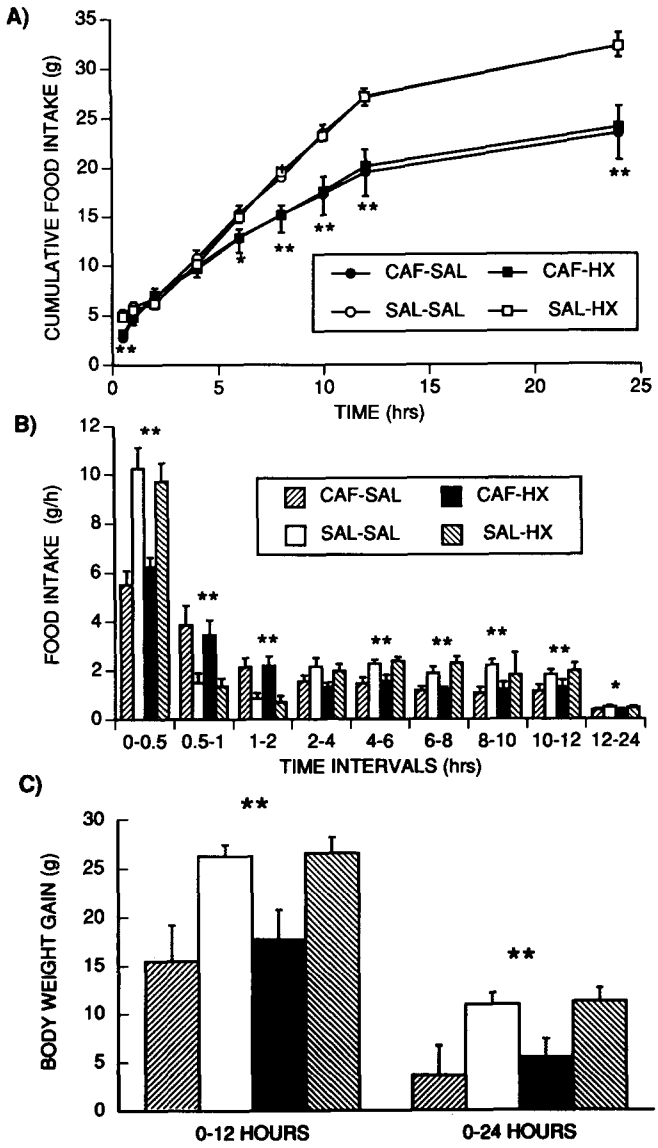

FIG. 4. Respective and interactive effects of caffeine and hexamethonium on cumulated (panel A) and noncumulated (panel B) food intake, and on body weight gain (panel C) in food-deprived rats (see the Method and Results sections for further details). Asterisks reveal a significant main effect of caffeine at the probability levels of 95 (*) or 99 (**) percent.

(panel B). However, because an enhanced intake was observed in medullectomized animals between 10–12 h, total food intake over the 24-h period postcaffeine was not changed by medullectomy. In addition, medullectomy had no effect on the body weight gains.

Ganglionic Blockade

Figure 4 depicts the respective and interactive influence of caffeine and hexamethonium on food intake and body weight gain in food-deprived rats. This series of experiments confirmed the anorectic effect of caffeine on cumulative food intake at several time points (panel A) and during various time intervals after the caffeine administration (panel B). There was no main effect of hexamethonium and no caffeine-hexamethonium interaction effect of food intake and body weight gains.

DISCUSSION

The present results clearly indicate that caffeine can reduce food intake and body weight gain in rats, therefore corroborating previous results obtained in humans (23). Two approaches were used to establish the anorectic effect of caffeine; caffeine was administered in either spontaneously fed or in food-deprived rats, and both these procedures revealed the effect of caffeine at various time points following its injection. The reduction of body weight gain produced by caffeine appears, at least partly, to be explained by its effect on food intake. The possibility that caffeine has also affected body weight by increasing energy expenditure cannot be ruled out, as caffeine may exert thermogenic actions (7). One intriguing finding obtained in this study is the two-phase hypophagic response to caffeine observed in food-deprived rats. In fact in these rats, the lowering effect ($p < 0.05$) of caffeine on food intake was apparent at 0.5 and 1 h after the caffeine administration (Fig. 1, panel B), then vanished for 3 h, and became significant again 6 h from the time caffeine was administered. On the basis of the present results, this biphasic response cannot be readily accounted for. However, the observation that α-helical $CRH_{(9-41)}$ may prevent only the later anorectic effect of caffeine suggest that the biphasic effect of caffeine on food intake involves two distinct mechanisms acting to induce an immediate and a later anorectic effect.

This study also points out a role for CRH in the effects of caffeine on food intake and body weight gain. As clearly illustrated in Fig. 2 and further revealed by the statistically significant caffeine-α-helical $CRH_{(9-41)}$ interactions, the use of a CRH antagonist largely prevented the effects of caffeine on food intake of food-deprived rats. The use of α-helical $CRH_{(9-41)}$ also blunted the effects of caffeine on the body weight gain. These results further emphasized the role of CRH in the control of food intake. Such a role of CRH has been previously characterized in various situations bringing about reductions of food intake. CRH neutralization either with antagonists or antibodies has been reported to attenuate the anorectic effects of exercise (19), restraint stress (22), estradiol (6), serotonin agonists (14), and interleukin 1 (24). It is worth pointing out that α-helical $CRH_{(9-41)}$ did not increase food intake in rats not receiving caffeine. This observation indicates that under basal conditions CRH is probably not involved in control of food intake. This finding does not, however, imply that the role of CRH is physiologically unsignificant; CRH has been shown to play a physiological role in the control of food intake under particular circumstances (19,22).

In contrast with the CRHergic system, the sympatho-adrenal system does not appear to be involved in the anorectic effects of caffeine. Experimental interventions such as the adrenal demedullation as well as the ganglionic blockade clearly failed to prevent the effects of caffeine on food intake and body weight gain. A role for the sympatho-adrenal axis in the anorectic effect of caffeine was expected on the basis that a stimulation of the sympatho-adrenal axis is liable of suppressing food intake through peripheral metabolic effects (12,21). In addition, the involvement of the sympathoadrenal axis in the effects of caffeine on food intake was worth considering from the suggestion that the anorectic action of CRH had been reported to imply the sympatho-adrenal system (4,9).

The reasons accounting for the lack of involvement of the sympatho-adrenal system in the CRH-mediated anorectic effects of caffeine on food intake remain obscure. It is, however, plausible that caffeine may lead to CRH effects on food intake that are independent from the sympatho-adrenal system. Endogenous CRH-releasers such as caffeine are susceptible to lead to more widespread and more persistent CRH effects on food intake than those induced by a single brain intracerebroventricular injections of CRH (9). Being widely distributed throughout the brain CRH may plausibly insure the control of food intake via populations of neurons not involved in governing the sympathoadrenal system.

In summary, the present results indicate that caffeine can reduce food intake and body weight gain in rats. In addition, these results, by providing evidence for a CRH-mediated anorectic action of caffeine further emphasized the role of CRH in the control of food intake. By emphasizing the inability of both the adrenal demedullation and the ganglionic blockade to prevent the CRH-mediated effects of caffeine, the present results provide evidence that the CRH effects on food intake do not obligatory involve the sympatho-adrenal system.

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REFERENCES

1. Astrup, A.; Toubro, S.; Cannon, S.; Hein, P.; Madsen, J. Thermogenic synergism between ephedrine and caffeine in healthy volunteers: A double-blind, placebo-controlled study. Metabolism 40:323-329; 1991.
2. Astrup, A.; Toubro, S.; Christensen, N. J.; Quaade, F. Pharmacology of thermogenic drugs. Am. J. Clin. Nutr. 55:246S-248S; 1992.
3. Bangsbo, J.; Jacobsen, K.; Nordberg, N.; Christensen, N. J.; Graham, T. E. Acute and habitual caffeine ingestion and metabolic responses to steady-state exercise. J. Appl. Physiol. 72: 1297-1303; 1992.
4. Britton, D. R.; Indyk, E. Effects of ganglionic blocking agent on behavioral responses to centrally administered CRF. Brain Res. 478:205-210; 1989.
5. Charney, D. S.; Heninger, G. R.; Jatlow, P. I. Increased anxiogenic effects of caffeine in panic disorders. Arch. Gen. Psychiatry 42:233-243; 1985.
6. Dagnault, A.; Ouerghi, D.; Richard, D. Treatment with α-helical-CRF-$_{(9-41)}$ prevent the anorectic effect of 17-β-estradiol. Brain Res. Bull. 32:689-692; 1993.
7. Dulloo, A. G.; Miller, D. S. The thermogenic properties of ephedrine/methylxanthine mixtures: Animal studies. Am. J. Clin. Nutr. 43:388-394; 1986.
8. Glowa, J. R.; Barrett, J. E.; Russell, J.; Gold, P. W. Effects of corticotropin releasing hormone on appetitive behaviors. Peptides 13:609-621; 1992.
9. Gosnell, B. A.; Morley, J. E.; Levine, A. S. Adrenal modulation of the inhibitory effect of corticotropin releasing factor on feeding. Peptides 4:807-812; 1983.
10. Graham, T. E.; Sathasivam, P.; MacNaughton, K. W. Influence of cold, exercise, and caffeine on catecholamine and metabolism in men. J. Appl. Physiol. 70:2052-2058; 1991.
11. Kirk, R. E. Experimental design: Procedures for the behavioral sciences. Pacific Grove: Brooks/Cole; 1982.
12. Langhans, W.; Scharrer, E. Metabolic control of eating. In: Simopoulos, A. P., ed. World review of nutrition and dietetics. Basel: Karger; 1992:1-67.
13. LeBlanc, J.; Jobin, M.; Côté, J.; Samson, P.; Labrie, A. Enhanced metabolic response to caffeine in exercise-trained human subjects. J. Appl. Physiol. 59:832-837; 1985.
14. LeFeuvre, R. A.; Aisenthal, L.; Rothwell, N. J. Involvement of corticotrophin-releasing factor (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds. Brain Res. 555:245-250; 1991.
15. MacNaughton, K. W.; Sathasivam, P.; Vallerand, A.; Graham, T. E. Influence of caffeine on metabolic responses of men at rest in 28 and 5°C. J. Appl. Physiol. 68:1889-1895; 1990.
16. Morley, J. E.; Levine, A. S. Corticotropin-releasing factor and ingestive behaviors. In: De Souza, E. B.; Nemeroff, C. B., eds. Corticotropin-releasing factor basic and clinical studies of a neuropeptide. Boca Raton: CRC; 1990:267-274.
17. Nicholson, S. A. Stimulatory effect of caffeine on the hypothalamo-pituitary-adrenocortical axis in the rat. J. Endocrinol. 122: 535-543; 1989.
18. Richard, D.; Labrie, A.; Rivest, S. Tissue specificity of SNS response to exercise in mice exposed to low temperatures. Am. J. Physiol. 262:R921-R925; 1992.
19. Rivest, S.; Richard, D. Involvement of corticotropin-releasing factor in the anorexia induced by exercise. Brain Res. Bull. 25: 169-172; 1990.
20. Russek, M.; Racotta, R. Possible participation of oro-, gastro, enterohepatic reflexes in preabsoptive satiation. In: Kare, M. R.; Brand, J. K., eds. Interaction of the chemical senses with nutrition. London: Academic; 1986:374-393.
21. Russek, M.; Vega, C.; Barrera, J.; Soto-Mora, L. M.; Langazorta, A.; Racotta, R. Anorexia elicited by different catecholamines in rats. Appetite 9:119-126; 1987.
22. Shibasaki, T.; Yamauchi, N.; Kato, Y.; Masuda, A.; Imaki, T.; Hotta, M.; Demura, H.; Oono, H.; Ling, N.; Shizume, K. Involvement of corticotropin-releasing factor in restraint stress-induced anorexia and reversion of the anorexia by somatostatin in the rat. Life Sci. 43:1103-1110; 1988.
23. Tremblay, A.; Masson, E.; Leduc, S.; Houde, A.; Després, J. P. Caffeine reduces spontaneous energy intake in men but not in women. Nutr. Res. 8:553-558; 1988.
24. Uehara, A.; Sekuya, C.; Takasaki, Y.; Namiki, M.; Armura, A. Anorexia induced by interleukin I: involvement of corticotropin-releasing factor. Am. J. Physiol. 257:R616-R617; 1989.