Pharmacology Biochemistry & Behavior, Vol. 15, pp. 735–738, 1981. Printed in the U.S.A.

The Effect of Prostaglandins (PGE_2 and $PGF_{2\alpha}$) on Food Intake in Rats

ALLEN S. LEVINE AND JOHN E. MORLEY

Neuroendocrine Research Laboratory, Minneapolis VA Medical Center, Minneapolis, MN 55417 and Division of Endocrinology, Departments of Medicine and Food Science and Nutrition University of Minnesota, Minneapolis and St. Paul

Received 15 April 1981

LEVINE, A. S. AND J. E. MORLEY. *The effect of prostaglandins (PGE_2 and $PGF_{2\alpha}$) on food intake in rats.* PHARMAC. BIOCHEM. BEHAV. 15(5) 735–738, 1981.—Intracerebroventricular administration of $PGF_{2\alpha}$and PGE_2 suppressed food intake in several feeding models. $PGF_{2\alpha}$ (20 μg) and PGE_2 (20 μg to 1 μg) suppressed food intake following a 24 hour starvation. $PGF_{2\alpha}$ (20 μg) and PGE_2 (20 μg) suppressed food intake following central administration of the feeding induces norepinephrine and muscimol. These prostaglandins also suppressed stress induced eating using the tail pinch model at doses of 20 μg, 10 μg and 5 μg with eating returning to control levels at the 1 μg dose. D-Ala Methionine Enkephalin failed to alter the suppressive effects of $PGF_{2\alpha}$ and PGE_2 at a dose of 1 μg but successfully reversed the effect of $PGF_{2\alpha}$ at a 10 μg dose while still having no effect on PGE_2 suppression of feeding.

Prostaglandins Food intake Stress induced eating Tail pinch

PROSTAGLANDINS are ubiquitously distributed throughout the body and are synthesized from fatty acid precursors by various tissues including brain [6,7]. In 1964, Horton reported that prostaglandins of the E series decreased feeding in starved cats after intracerebroventricular injection [18]. Subsequently, Baile and co-workers demonstrated that a variety of prostaglandins (E_1, E_2, $E_{1\alpha}$, $E_{2\alpha}$, A_1, B_1) decreased feeding in the rat (after parenteral and/or intrahypothalamic injections [4,32]) and in sheep after intrahypothalamic injections [3]. Doggett and Jawaharlal [10] demonstrated that a number of prostaglandin precursors inhibit food intake in the rat and this anorexia can be reversed by indomethacin. Recently, PGB_x, an oligomeric derivative of prostaglandin B_1 has been shown to decrease appetite and to normalize weight and blood glucose in hereditary diabetic mice [31]. Prostaglandins produce their anorectic activity without producing behavioural depressant effects [4]. The anorectic effect is not related to changes in temperature since the satiety effect is seen after administration of prostaglandins that produce either hypo- or hyperthermia [4,10]. Also Doggett and Jawaharlal [10] showed that the anorexia is not due to pain or irritative properties of prostaglandin injections since induction of comparable pain with 3% acetic acid did not affect food intake in rats deprived of food for 22 hours. PGE_1 has been shown to be effective at reducing food intake when injected into the lateral hypothalamic and anterior commisural, but not in the perifornical hypothalamic area [4]. In sheep, PGE_1 has a dual action on food intake; reducing feeding when injected in sites in the hypothalamus where norepinephrine induces feeding and eliciting feeding in the lateral hypothalamic, β-adreno-receptor sensitive areas [3].

Baile *et al.* [4] have suggested that the prostaglandins may be components of a signal relating fat depots and energy balance regulation. They hypothesized that prostaglandins produced in adipose tissue acts on hypothalamic centers to modulate long term control of feeding and thus play a role in the maintenance of energy balance. Evidence in favor of their hypothesis came from the finding that in man, fasting is associated with lower prostaglandin levels in blood compared to post-prandial samples [15]. The studies suggesting a role of insulin in long term regulation in appetite [5,22] would be compatible with the Baile hypothesis as insulin is closely associated with the formation of polyunsaturated fatty acids which are prostaglandin precursors.

We have suggested that the hypothalamus acts as a neuroendocrine transducer with the control of food intake involving a delicate balance between a number of neuropeptides and monoamines [23]. Based on the above information, it seems highly likely that prostaglandins interact with monoamines and peptides responsible for producing the integrated regulation of appetite. In this study we report the effects of PGE_2 and $PGF_{2\alpha}$ on a variety of feeding models. The studies reported here allow us to integrate the prostaglandins into our previously proposed hypothesis of the interrelationships of monoaminergic and peptidergic substances involved in the intrahypothalamic regulation of appetite [23,28].

METHOD

Male Sprague-Dawley rats (200–250 g) kept under standard lighting conditions (12 hr/day artificial light—0700–1900 hr) and given free access to a standard rat diet and water, were used for all experiments. Cannulas were implanted into the lateral ventricles as previously described [25]. The animals were allowed a minimum of 5 days post-operative recovery before experiments were commenced. Placement of cannula was checked at post mortem by indian ink injection.

The food deprivation experiments were conducted using

TABLE 1
EFFECT OF $PGF_{2\alpha}$ ON STARVATION INDUCED EATING

		Food Intake	
	n	g/30 min	g/60 min
Vehicle	14	1.4 ± 0.2	1.7 ± 0.2
$PGF_{2\alpha}$ 20 μg	9	0.1 ± 0.1*	0.8 ± 0.2*
10 μg	6	1.5 ± 0.3	1.6 ± 0.3
5 μg	6	1.4 ± 0.3	1.4 ± 0.3
$PGF_{2\alpha}$ (20 μg)+DAlaMetEnkephalin 1 μg	10	0.4 ± 0.2	0.9 ± 0.2
$PGF_{2\alpha}$ (20 μg)+DAlaMetEnkephalin 10 μg	5	1.7 ± 0.8‡	2.6 ± 0.5†

*$p<0.01$ vs vehicle; †$p<0.01$ and ‡$p<0.05$ vs $PGF_{2\alpha}$ 20 μg.

rats which were starved for 24 hours (water ad lib). Immediately after intracerebroventricular administration of PGE_2 (20, 10, 5, 1, 0.1, 0.01 μg/5 λ), $PGF_{2\alpha}$ (20, 10, 5 μg/5 λ), D-Ala-Met-Enkephalin (1, 10 or 20 μg/5 λ) or vehicle 15% ethanol, 0.3% Na_2CO_3, 0.9% NaCl (5 λ)) the rats were placed into unfamiliar plastic boxes containing 2 pellets of pre-weighed Purina rat chow (7–10 g). Animals were allowed free access to the food for 30 minutes at which time the original pellets and spillage were removed and they were given two more pellets for a further 30 minutes. Food ingestion was expressed as grams eaten/30 or 60 minutes.

The effect of prostaglandins on stress-induced eating as measured using the tail-pinch model of Antelman [2] was also assessed. We used the tail-pinch method as modified by us [25]. Tail-pinch was induced with a plastic clamp (1.7 cm wide at the tip: MacBick Co., Murray Hill, NJ) which gives good control of the range of pressures that can be exerted compared to the surgical hemostat. Behavoral testing was carried out in an unfamiliar 22×17 cm plastic box, containing two pellets of Purina rat chow (6–10 g). In the majority of animals, tail-pinch behavior is induced before the onset of pain (as indicated by vocalization). The mild tail-pinch is applied for a period of 2 minutes. Food ingestion is quantitated by carefully weighing the pellet before and after the experimental period. The prostaglandins or vehicle were then administered ICV and the mild tail-pinch repeated 15 minutes later for a 2 minute period. Results were expressed as:

$$\frac{\text{Amount of food ingested after drug}}{\text{Amount of food ingested before drug}} \times 100$$

Norepinephrine (20 μg) was freshly dissolved in slightly acidified saline and administered in a 5 μl volume ICV. PGe_2 and $PGF_{2\alpha}$ were administered immediately prior to norepinephrine in a 5 μl volume ICV. All animals had free access to food and water until the experiments were performed. Immediately after drug administration, animals were put in a new cage together with 2 pellets of pre-weighed Purina rat chow (7–10 g). Food intake is expressed as grams eaten/30 minutes.

The GABA agonist, muscimol, (500 ng) was administered ICV in a 5 μl volume. All other aspects of the muscimol induced feeding study were identical to the norpinephrine study with the exception that food intake was calculated for two 30 minute periods after injection.

All results were expressed as the mean±S.E.M. Results were compared using the two-tailed Student's *t*-test. PGE_2, $PGF_{2\alpha}$, norepinephrine, and muscimol were obtained from Sigma Chemical Company, St. Louis, MO: and D-Alanine-Methionine-Enkephalin from Boehringer Mannheim Biochemicals, Indianapolis, IN. The highest doses of the various drugs were doses previously shown to produce reproducible effects on feeding behavior.

RESULTS

$PGF_{2\alpha}$ (Table 1) and PGE_2 (Table 2) in a 20 μg dose suppressed food intake in food deprived animals for 30 and 60 minutes after administration. PGE_2 suppressed food intake at doses as low as 1 μg whereas the suppressive effect of $PGF_{2\alpha}$ was no longer present at the 10 and 5 μg doses. D-Ala-Met-Enkephalin (1 μg) failed to reverse the suppressive effect of these two prostaglandins on food intake, whereas 10 μg reversed the suppressive effect of $PGF_{2\alpha}$.

Stress-induced eating as quantitated by the mild tail-pinch model was suppressed by PGE_2 and $PGF_{2\alpha}$ over a dose range of 20 μg, 10 μg, and 5 μg with eating returning to control levels at the 1 μg dose (Table 3). All animals displayed normal chewing behavior during tail-iinch suggesting that prostaglandins do not produce a generalized disruption of behavior.

Both prostaglandins decreased norepinephrine-induced feeding (Fig. 1) and muscimol-induced feeding (Fig. 2).

DISCUSSION

In this study we have demonstrated that both PGE_2 and $PGF_{2\alpha}$ suppress food intake in a variety of feeding models. It is possible that $PGF_{2\alpha}$ is the most important prostaglandin involved in the intrahypothalamic regulation of feeding as it is a natural constituent of all the mammalian brains studied [16] and the F series of prostaglandis is the only one that has been demonstrated to be synthesized in rat brain [11]. The fact that in the starvation model only the highest dose of $PGF_{2\alpha}$ suppressed eating, mitigates against the possible physiological importance of $PGF_{2\alpha}$. It is possible that prostaglandins of the E series, which have been shown to have anorectic activity when administered parenterally [32], may play a role as peripheral signals involved in the energy balance regulation system as suggested by Baile *et al.* [4]. Alternatively, the fatty acid precursors of the prostaglandins (which have been reported to cross the blood-brain barrier

TABLE 2

EFFECT OF PGE_2 ON STARVATION INDUCED EATING

	n	Food Intake g/30 min	Food Intake g/60 min
Vehicle	14	1.8 ± 0.4	2.0 ± 0.4
PGE_2 20 μg	10	0.8 ± 0.3†	1.2 ± 0.2†
10 μg	6	0.6 ± 0.2*	0.7 ± 0.3†
5 μg	6	0.4 ± 0.1*	0.7 ± 0.2†
1 μg	7	0.7 ± 0.3†	0.8 ± 0.2†
0.1 μg	7	1.1 ± 0.3	1.2 ± 0.4
0.01 μg	8	1.8 ± 0.1	1.9 ± 0.2
PGE_2 (20 μg)+DAlaMetEnkephalin 1 μg	7	0.5 ± 0.2	0.9 ± 0.4
PGE_2 (20 μg)+DAlaMetEnkephalin 10 μg	6	0 ± 0	0.6 ± 0.3

*$p<0.01$ and †$p<0.05$ vs vehicle.

TABLE 3

EFFECT OF PGE_2 AND $PGF_{2\alpha}$ ON MILD TAIL-PINCH INDUCED EATING

	n	% Control	p
Vehicle	8	128 ± 12	
PGE_2 20 μg	8	41 ± 10	<0.001
10 μg	8	30 ± 11	<0.001
5 μg	8	75 ± 19	<0.05
1 μg	7	96 ± 31	N.S.
$PGF_{2\alpha}$ 20 μg	6	17 ± 4	<0.001
10 μg	6	63 ± 19	<0.01
5 μg	6	61 ± 21	<0.01
1 μg	8	103 ± 31	N.S.

Basal food intake was 0.5 ± 0.03 g/2 min.

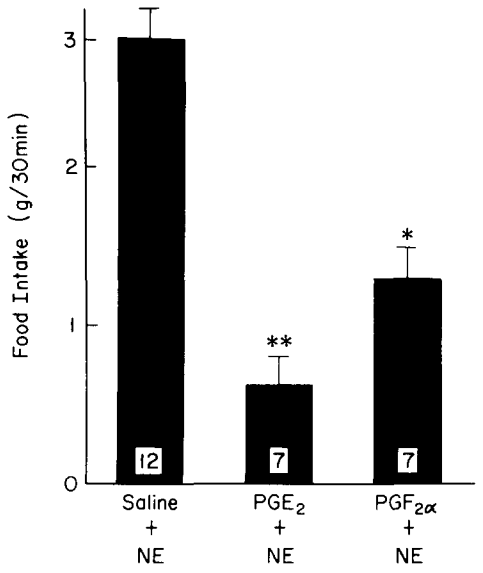

FIG. 1. The effect of PGE_2 (20 μg) and $PGF_{2\alpha}$ (20 μg) on norepinephrine (20 μg)-induced feeding. **$p<0.001$, *$p<0.01$.

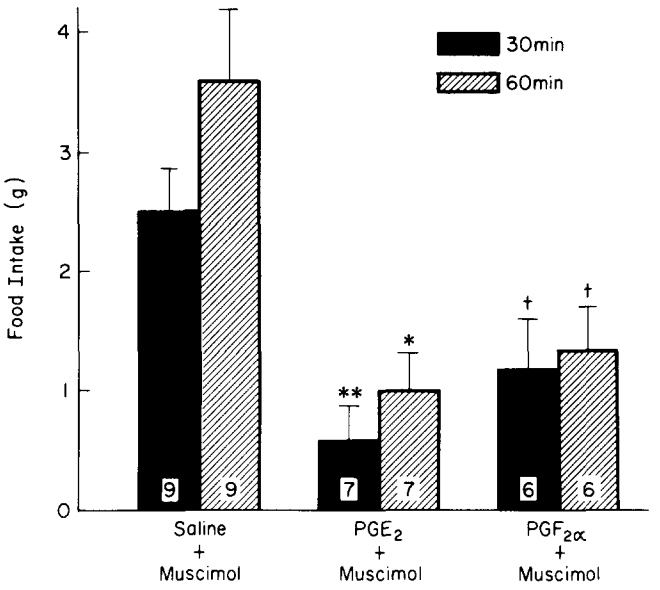

FIG. 2. The effect of PGE_2 (20 μg) and $PGF_{2\alpha}$ (20 μg) on muscimol (500 ng)-induced feeding. **$p<0.001$, *$p<0.01$, †$p<0.05$.

[1]) may serve as peripheral signals by activating the prostaglandin generating system.

A number of lines of evidence suggest that the endogenous opiates are intimately related to the production of the feeding drive in the hypothalamus (reviewed in [23]). The endogenous opiate antagonist, naloxone, suppresses eating in a variety of situations [8, 17, 19, 21, 23, 24] and intrahypothalamic injection of β-endorphin initiates feeding in sated animals [14]. We have shown that one microgram of D-Ala-Met-Enkephalin, a long acting methionine-enkephalin analog, administered ICV reverses the anorectic effect of thyrotropin-releasing hormone, histidyl-proline diketopiperazine, bombesine and cholecystokinin ([25,27] and unpublished observations). PGE_1 has been shown to interact with the endogenous opiates, viz. PGE_1 antagonizes opiate effects in the neuroblastoma cell line [9]. In the present

study, we examined whether the anorectic effect of PGE_2 and $PGF_{2\alpha}$ could be altered by D-Ala-Met-Enkephalin.

The failure of D-Ala-Met-Enkephalin to modulate the satiety effect of PGE_2 suggests that PGE_2 may play a role as a terminal endogenous feeding inhibitor in the lateral hypothalamus. The intrahypothalamic injection experiments localizing PGE_1 effects to the lateral hypothalamus of the rat would support this concept [4]. The ability of the 10 μg dose of D-Ala-Met-Enkephalin to reverse the anorectic effect of $PGF_{2\alpha}$ but not that of PGE_2 is a more potent anorectic substance.

The mild tail-pinch method of producing stress-induced eating represents an excellent screening test for anorectic peptides [20]. Both PGE_2 and $PGF_{2\alpha}$ inhibited tail pinch induced eating in a dose dependent manner. As the rats maintained normal chewing behavior after prostaglandins this suggests that the effect of prostaglandins is not secondary to non-specific disruption of behavior. The tail-pinch model has been demonstrated to involve the activation of endogenous opiates [21,24]. The ability of the centrally administered prostaglandins to inhibit stress-induced feeding provides further evidence suggesting that the prostaglandins may induce satiety by inhibiting endogenous opiate activity.

We have previously shown [28] that the putative satiety factors, bombesin [13,26], cholecystokinin[12,30] and thyrotropin-releasing hormone [25,33] do not suppress feeding induced by the GABA-agonist, muscimol. Thus it appears that the prostaglandins join calcitonin as the only two putative satiety factors that suppress muscimol induced feeding [28]. As norepinephrine induced feeding is thought to be secondary to activation of the GABAergic system in the venteromedial hypothalamus [14,29] it was not surprising to find that the prostaglandins blocked norepinephrine-induced eating as well.

REFERENCES

1. Alfin-Salter, R. B. and L. Aftergood. Essential fatty acids reinvestigated. *Physiol. Rev.* **48:** 758–784, 1968.
2. Antelman, S. M. and H. Szechtman. Tail-pinch induces eating in sated rats which appear to depend on nigrostriatal dopamine system. *Science* **198:** 731–733, 1975.
3. Baile, C. A., F. H. Martin, J. M. Forbes, R. L. Webb and W. Kingsbury. Intrahypothalamic injections of prostaglandins and prostaglandin antagonists and feeding in sheep. *J. Dairy Sci.* **57:** 81–88, 1974.
4. Baile, C. A., C. W. Simpson, S. M. Bean, C. L. McLaughlin and H. L. Jacobs. Prostaglandins and food intake of rats: A component of energy balance regulation? *Physiol. Behav.* **10:** 1077–1085, 1973.
5. Benjamin, W. and A. Gellhorn. The effect of diabetes and insulin in the biosynthesis of individual fatty acids in adipose tissue. *J. biol. Chem.* **239:** 64–69, 1964.
6. Bergström, S., H. Danielsson and B. Samuelsson. The enzymatic formation of prostaglandin E_2 from arachidonic acid. *Biochim. biophys. Acta* **90:** 207–210, 1964.
7. Bergström, S., H. Danielsson, D. Klenberg and B. Samuelsson. The enzymatic conversion of EFA into prostaglandins. *J. biol. Chem.* **239:** 4006–4008, 1964.
8. Brands, B., J. A. Thornhill, M. Hirst and C. W. Gowdey. Suppression of food intake and body weight gain by naloxone in rats. *Life Sci.* **24:** 1773–1778, 1979.
9. Dawson, G., R. McLawhon and R. J. Miller. Inhibition of sialoglycosphingolipid (ganglioside) biosynthesis in mouse clonal lines N4TG1 and NG108–15 by β-endorphins, enkephalins and opiates. *J. biol. Chem.* **255:** 129–137, 1980.
10. Doggett, N. S. and K. Jawaharlal. Anorectic activity of prostaglandin precursors. *Br. J. Pharmac.* **60:** 417–423, 1977.
11. Feldberg, W. Fever, prostaglandins and antipyretics. In: *Prostaglandin Synthetase Inhibitors*, edited by H. J. Robinson and J. R. Vane. New York: Raven Press, 1974, pp. 197–203.
12. Gibbs, J., R. C. Young and G. P. Smith. Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* **245:** 323–325, 1973.
13. Gibbs, J., D. J. Fauser, E. A. Rowe, B. J. Rolls, E. T. Rolls and S. P. Maddison. Bombesin suppresses feeding in rats. *Nature* **282:** 208–210, 1979.
14. Grandison, L. and A. Guidotti. Stimulation of food intake by muscimol and beta endorphin. *Neuropharmacology* **16:** 533–536, 1977.
15. Greaves, M. W., W. J. McDonald-Gibson and R. G. McDonald-Gibson. The effect of venous occlusion, starvation and exercise on prostaglandin activity in whole human blood. *Life Sci.* **11:** 919–924, 1972.
16. Holmes, S. W. and E. W. Horton. Prostaglandins and the central nervous system. In: *Prostaglandin Symposium of the Worcester Foundation for Experimental Biology*, edited by P. Ramwell and J. E. Shaw. New York: Wiley, 1968, pp. 21–38.
17. Holtzman, S. G. Suppression of appetite behavior in the rat by naloxone: Lack of effect of prior morphine dependence. *Life Sci.* **24:** 219–226, 1979.
18. Horton, E. W. Actions of prostaglandins E_1, E_2 and E_3 on the central nervous system. *Br. J. Pharmac. Chemother.* **22:** 189–192, 1964.
19. King, B. M. F. X. Castellanos, A. J. Kastin, M. C. Berzas, M. D. Mauk, G. A. Olson and R. D. Olson. Nalxone-induced suppression of food intake in normal and hypothalamic obese rats. *Pharmac. Biochem. Behav.* **11:** 729–732, 1979.
20. Levine, A. S. and J. E. Morley. Stress-induced eating in rats. *Am. J. Physiol.* **241:** R72–R76, 1981.
21. Lowy, M. T., R. P. Maickel and G. K. W. Yim. Naloxone reduction of stress-related feeding. *Life Sci.* **26:** 2113–2118, 1980.
22. Mercuri, O., R. O. Peluffo and R. R. Brenner. Depression of microsomal desaturation of linoleic to δ-linoleic acid in the alloxan-diabetic rat. *Biochim. biophys. Acta* **116:** 409–411, 1966.
23. Morley, J. E. The neuroendocrine control of appetite: The role of the endogenous opiates, cholecystokinin, TRH, gamma amino butyric acid and the diazepam receptor. *Life Sci.* **27:** 355–368, 1980.
24. Morley, J. E. and A. S. Levine. Stress induced eating is mediated through endogenous opiates. *Science* **209:** 1259–1261, 1980.
25. Morley, J. E. and A. S. Levine: Thyrotropin releasing hormone (TRH) suppresses stress-induced eating. *Life Sci.* **27:** 269–274, 1980.
26. Morley, J. E. and A. S. Levine. Bombesin suppresses stress-induced eating. *Pharmac. Biochem. Behav.* **14:** 149–151, 1981.
27. Morley, J. E. and A. S. Levine. Histidyl-proline diketopiperazine decreases food intake in rats. *Brain Res.* **210:** 475–478, 1981.
28. Morley, J. E., A. S. Levine and J. Kneip. Muscimol induced feeding: A model to study the hypothalamic regulation of appetite. *Life Sci.* **29:** 1213–1218, 1981.
29. Morley, J. E., A. S. Levine, S. S. Murray and J. Kneip. Peptidergic regulation of norepinephrine induced feeding. *Pharmac. Biochem. Behav.*, in press.
30. Nemeroff, C. B., A. J. Osbahr, G. Bissette, G. Jahnke, M. A. Lipton and A. J. Prange. Cholecystokinin inhibits tail pinch induced eating in rats. *Science* **200:** 793–794, 1978.
31. Polis, B. D. and E. Polis. Dose dependence of PBG_s, a polymeric derivative of prostaglandin B_1, for normalization of hereditary diabetes of the mouse. *Physiol. Chem. Physics* **11:** 3–8, 1979.
32. Scaramuzi, O. E., C. A. Baile and J. Mayer. Prostaglandins and food intake of rats. *Experientia* **27:** 256–257, 1971.
33. Vijayan, E. and S. M. McCann. Suppression of feeding and drinking activity in rats following intraventricular injection of thyrotropin releasing hormone. *Endocrinology* **100:** 1727–1730, 1977.