Pharmacology Biochemistry & Behavior, Vol 24, pp 899–903, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Opioid and Non-Opioid Components of Insulin-Induced Feeding

E. C. C. SI, H. U. BRYANT AND G. K. W. YIM[1]

Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences Purdue University, West Lafayette, IN 47907

Received 22 July 1985

SI, E C C, H U BRYANT AND G K W YIM *Opioid and non-opioid components of insulin-induced feeding* PHARMACOL BIOCHEM BEHAV 24(4) 899–903, 1986 —The present study was initiated to clarify the involvement of endogenous opioids in insulin-induced feeding. Naloxone (3 mg/kg) was injected in male Sprague Dawley rats every hour for 2 hours after insulin injection (10 U/kg) Only the first hour food intake was depressed (68% reduction). When naloxone was given only 1 hour after insulin administration, depression of food intake was not noted When food was withheld for 2 hours after insulin injection, both naloxone and its long acting congener, naltrexone (3 mg/kg) were able to depress only the first hour feeding subsequent to food presentation These data suggest that insulin-induced feeding can be divided into two pharmacologically distinct phases. the early phase being naloxone-sensitive while the late phase is naloxone-insensitive Furthermore, the early phase begins with the presentation of food and not with the administration of insulin

Naloxone Naltrexone Insulin Food intake Endogenous opioids

PERIPHERAL administration of insulin causes an increase in food intake in a variety of species [9, 11, 18]. Although a direct effect on the insulin receptor in the ventral-medial hypothalamus is suspected [23], insulin-induced eating is often attributed to the central glucoprivation resulting from increased glucose uptake into the non-neural tissue caused by insulin. This is further supported by the fact that 2-deoxy-D-glucose (2-DG), a glucoprivic agent, also increases food intake. However, recent evidence indicates that 2-DG and insulin-induced feeding might not be mediated by the same mechanism. For instance, various brain lesions and surgical manipulations can differentially attenuate one, but not the other type of glucoprivic feeding [10]. This has led to a number of studies that attempt to differentiate between these two types of feeding [15,24].

It is well established that 2-DG-induced feeding is mediated via an opioid mechanism Naloxone, an opiate antagonist, was shown to reduce the 3 hour feeding response induced by 2-DG in a dose-related fashion [8]. The effect of opiate antagonists on insulin-induced feeding is less clear Lowy *et al.* [8] reported that high doses of naloxone (up to 16 mg/kg) failed to block the 3 hour insulin-induced feeding response However, Ostrowski *et al* [15] and Levine and Morley [7] later reported that high doses of naloxone (up to 20 mg/kg) did suppress the 1 hour, but not 2 or 3 hour feeding following insulin administration.

One difficulty in interpreting the effects of naloxone at the early time points or the later time points of insulin-induced eating is the relatively short half-life of naloxone (approx. 30–40 min in rats [13]) Thus, there could be a pharmacokinetic explanation for the lack of naloxone effect in the late phase. In an attempt to circumvent this difficulty, naltrexone, a long acting opiate antagonist was used in an earlier study [24]. It marginally (not statistically significant) reduced the first hour insulin-induced feeding. Hence, a more direct approach is needed.

Another problem lies in the dosage of naloxone The doses of naloxone used to suppress insulin-induced feeding are relatively high when compared to those that are effective in suppressing food deprivation-induced eating or other types of opiate-sensitive hyperphagia [8,12].

The present study used a relatively low dose of naloxone (3 mg/kg) to determine whether there exists different phases of insulin-induced feeding with regard to opiate-sensitivity A multiple dosing regimen of naloxone was used to test the pharmacokinetic explanation for the failure of naloxone to suppress the insulin-induced feeding at the later time points.

METHOD

Male mice (Swiss-Webster, 25–35 g) and male rats (Sprague-Dawley, 300–400 g) were obtained from Laboratory Supply Co. (Indianapolis, IN). Mice were housed in pairs in plastic containers with stainless steel tops (47×25 cm) Rats were housed individually in stainless steel cages (25×21×20 cm) Animals were handled and given saline injections in four preliminary sessions to habituate them to experimental procedures. The animals had free access to water and Wayne Lab Blox placed on the cage floor Rats and mice were housed in separate rooms at 22°C on an alternating light-dark cycle (L 0800–2000; D· 2000–0800).

All experiments were initiated between 1000 hour and

[1]Requests for reprints should be addressed to G K W. Yim.

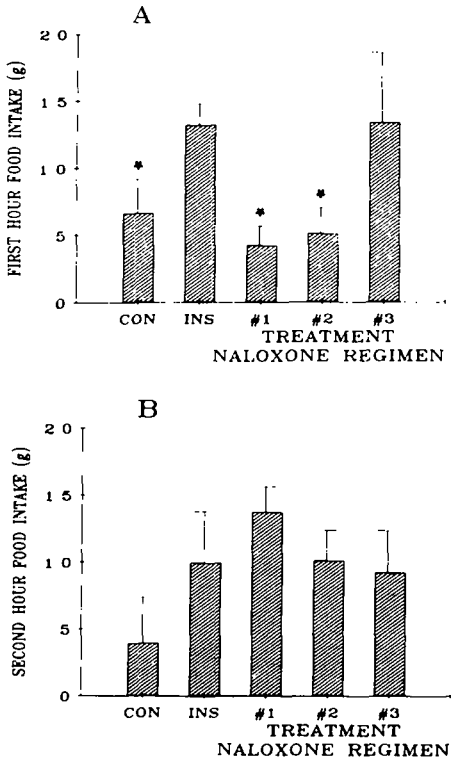

FIG 1 First hour (A) and second hour (B) of the insulin (INS)-induced feeding with 3 dosing regimens of naloxone (NAL) Treatment 1—NAL (3 mg/kg) was injected 10 minutes after INS (10 U/kg) injection Treatment 2—NAL was injected 10 minutes and again 1 hour after INS injection Treatment 3—NAL was injected 1 hour after INS injection Control (CON) animals were treated with saline Bars represent mean 1 hour food intake (g) from 6 rats +S E *=$p<0$ 05, vs insulin group

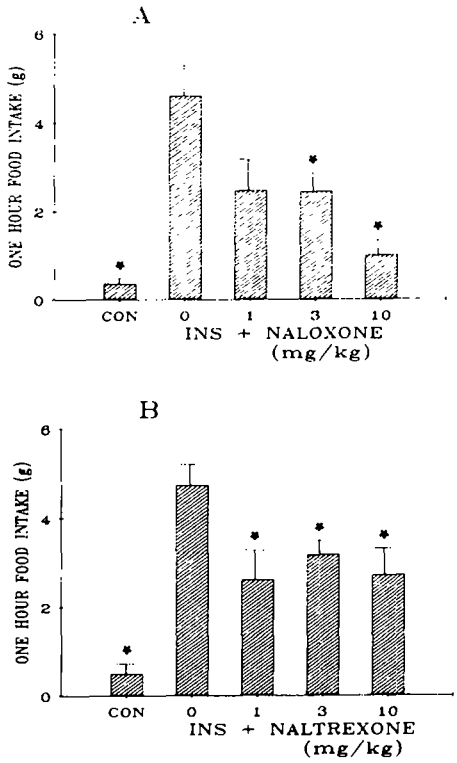

FIG 2 Effect of naloxone (A) and naltrexone (B) on insulin (INS)-induced feeding Food was withheld for 2 hours following injection of INS (10 U/kg) or saline (CON) Bars represent mean of the subsequent 1 hour food intake (g) from 6 rats +S E *=$p<0$ 05, vs insulin group

1200 hour. In the multiple dosing experiment, rats were divided into five groups each with six animals. The first two groups were injected with saline and insulin respectively. The third group of animals were injected with 3 mg/kg naloxone SC 10 min after administration of insulin (treament 1) This treatment duplicates the experiments done by Ostrowski *et al* [15] and Levine and Morley [7] but with a lower dose of naloxone. The fourth group of animals were treated with naloxone 10 min and again 1 hour after the administration of insulin (treatment 2) This procedure should determine whether the failure of naloxone to suppress the second hour food intake by insulin is caused by its short half-life The last group of animals were treated with naloxone 1 hour after the administration of insulin (treatment 3). All insulin-treated animals were injected with a dose of 10 U/kg unless otherwise stated Immediately after the injections, rats were returned to their home cages where preweighed quantities of food were placed on the cage floor. Food intake was measured at 1, 2, 4, and 6 hours following presentation of food by subtracting spillage on paper towels and uneaten food from the original quantity

The next experiment was initiated to further study the phasic nature of insulin-induced feeding Rats were injected with insulin and were immediately returned to their home cages Two hours later, naloxone or naltrexone was administered SC in a dosage range of 1 to 10 mg/kg A premeasured quantity of food was then presented Food intake was measured at 1, 2, 4, and 6 hours after food presentation

A final experiment was initiated to extend the observations in rats to mice Insulin (5 U or 10 U/kg, SC) was administered to pairs of mice. Ten min later, naloxone (5 or 10 mg/kg) was injected. Pooled food intake from a pair of mice was measured 1 and 2 hours after food presentation.

Unless otherwise specified, all results were compared using analysis of variance and post-hoc Newman-Keuls tests where indicated.

RESULTS

The results of the multiple dosing experiment with naloxone are presented in Fig 1 During the first hour of feeding following insulin administration, food intake was reduced ($p<0.05$) in both treatment groups receiving naloxone (treatments 1 and 2, Fig. 1A) by 68% and 61% respectively Feeding in treatment group 3, which received only saline pre-administration, was unaffected. Interestingly, naloxone had no suppressant effect on appetite when given just prior to the second hour of insulin-induced feeding (Fig 1B), either in animals who had prior administration of naloxone (treatment 2) or in those who had not (treatment 3) Therefore, the theoretical maintenance of naloxone blood levels through multiple dosing did not perpetuate the reduction in

TABLE 1

CUMULATIVE FOOD INTAKE (g) BY RATS GIVEN INSULIN OR SALINE AND THEN INJECTED WITH VARIOUS DOSES OF NALOXONE IN A 2 HOUR DELAY FEEDING PARADIGM

	Time After Food Presentation			
Treatment	1 hour	2 hour	4 hour	6 hour
Saline	0 37 ± 0 21*†	0 85 ± 0 42*	1 38 ± 0 45*	2 83 ± 0 86*
Insulin	4 62 ± 0 68	4 87 ± 0 73	4 95 ± 0 70	5 80 ± 0 44
Insulin + Naloxone (1 mg/kg)	2 48 ± 0 69	2 50 ± 0 69	4 02 ± 0 60	4 60 ± 1 00
Insulin + Naloxone (3 mg/kg)	2 46 ± 0 40*	3 65 ± 0 56	4 33 ± 0 60	6 78 ± 0 82
Insulin + Naloxone (10 mg/kg)	1 02 ± 0 32*	1 77 ± 0 61*	2 45 ± 0 60*	4 37 ± 0 99

*$p<0.05$ vs insulin group
†Mean ± Standard Error of the Mean for 6 rats

TABLE 2

CUMULATIVE FOOD INTAKE (g) BY RATS GIVEN INSULIN OR SALINE AND THEN INJECTED WITH VARIOUS DOSES OF NALTREXONE IN A 2 HOUR DELAY FEEDING PARADIGM

	Time After Food Presentation			
Treatment	1 hour	2 hour	4 hour	6 hour
Saline	0 5 ± 0 22*†	0 97 ± 0 48*	2 37 ± 0 86*	4 22 ± 0 91*
Insulin	4 75 ± 0 56	4 93 ± 0 53	5 58 ± 0 63	7 27 ± 0 79
Insulin + Naltrexone (1 mg/kg)	2 62 ± 0 65*	3 53 ± 0 65	4 08 ± 0 70	5 82 ± 0 81
Insulin + Naltrexone (3 mg/kg)	3 18 ± 0 31*	3 80 ± 0 45	4 40 ± 0 82	5 87 ± 1 06
Insulin + Naltrexone (10 mg/kg)	2 73 ± 0 58*	3 30 ± 0 58	3 73 ± 0 66	6 33 ± 0 87

*$p<0.05$ vs insulin group
†Mean ± Standard Error of the Mean for 6 rats

food intake, and the same dose of naloxone which inhibited insulin-induced feeding in the first hour did not inhibit it in the second hour. When 2, 4 or 6 hour cumulative food intake was assessed, there were no significant reductions in any naloxone treatment group relative to the insulin treatment group.

When food was withheld for 2 hours following insulin injection in an attempt to bypass the early opiate-sensitive phase, naloxone retained its appetite suppressive activity (Fig. 2A). Following the 2 hour delay, insulin produced a strong, twelve-fold increase in subsequent 1 hour food intake. Both the 3 0 and 10 0 mg/kg doses of naloxone reduced ($p<0.05$) this effect during the first hour that food was made available by 47% and 79% respectively The high, 10 mg/kg dose of naloxone continued to depress cumulative 2 and 4 hour insulin-induced hyperphagia by 63% and 50% respectively (Table 1) By 6 hours (8 hours post-insulin injection), food intake was not significantly decreased in any of the naloxone treatment groups

Using a similar paradigm, naltrexone, a longer acting opiate antagonist, also suppressed insulin-induced feeding (Fig. 2B). Again, insulin induced a strong hyperphagic response (over nine-fold) in the first hour following the 2 hour delay. All three doses of naltrexone (1.0, 3.0 and 10.0 mg/kg) attenuated this response ($p<0.05$) during the first hour by 45%, 33% and 43% respectively. The effect however, was only observable during the first hour as at subsequent time points (2, 4 and 6 hour) cumulative food intake was not reduced (Table 2).

The sensitivity of only the first hour of insulin-induced feeding to opiate receptor blockade with naloxone was also observed in mice (Fig. 3). Five or 10.0 U/kg insulin resulted in roughly a two-fold increase in 1 hour food intake in the mice. Both 5.0 and 10.0 mg/kg naloxone reduced this effect ($p<0.05$) Cumulative 2 hour food intake was only suppressed by the 10.0 mg/kg dose of naloxone in the 10 0 U/kg insulin treatment group (42% reduction, data not shown), while 3, 4 and 6 hour cumulative food intakes were not suppressed in any of the naloxone treatment groups

DISCUSSION

The results of these experiments indicate the existence of pharmacologically separable phases of feeding subsequent to

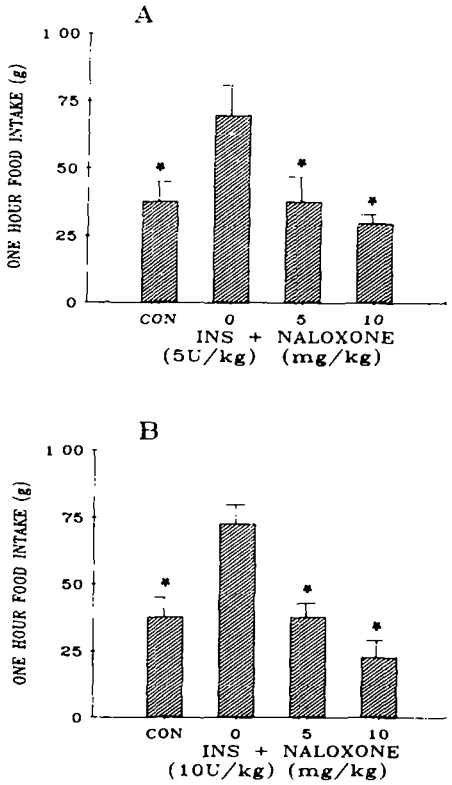

FIG 3 Effect of naloxone on food intake induced by 5 U/kg insulin (INS) (A) or 10 U/kg insulin (B) in mice Control (CON) animals were treated with saline Bars represent mean 1 hour food intake (g) from 6 pairs of mice +S E *=$p<0$ 05, vs insulin group

the SC injection of insulin These data show that naloxone suppresses only the first hour of feeding induced by insulin in both rats and mice This finding is in agreement with those of Levine and Morley [18], but with a lower dose The results of the multiple dosing regimen used in this study shows that the failure of naloxone to block insulin-induced food intake at later time points is not, as suggested by Rowland and Bartness [19], due to its short half-life. Rather, it may be due to the phasic nature of insulin-induced feeding. These data, along with the finding that naloxone does not attenuate insulin-induced feeding when given during the second hour, is compatible with the existence of a naloxone sensitive opiate component in the first hour of insulin-induced feeding followed by a late phase that is opiate independent The duration of effect of naloxone was equal to that of naltrexone in the second set of experiments, as both blocked only the first hour of insulin-induced feeding Depression of cumulative 2 and 4 hour feeding with naloxone was due to the inability of the animals to compensate for the first hour feeding deficit. Since naltrexone is a longer acting opiate antagonist than naloxone [1], this is further evidence that the lack of attenuation of insulin-induced feeding at the later time points cannot be explained by the pharmacokinetic properties of naloxone

When food was withheld for 2 hours following insulin injection, the early, naloxone-sensitive component remained intact Both naloxone and its longer acting congener, naltrexone, suppressed only the subsequent 1 hour feeding These data suggest that the two phases begin with the presentation of food and not with the administration of insulin Many studies have linked the reward associated with food intake to endogenous opioids [2,5] It is possible then that opiate antagonists interrupt early insulin-induced feeding by interfering with the reward associated with that feeding At later time points, insulin-induced feeding may not be as dependent on the reward associated with the act of feeding

The effect of naloxone on hypoglycemia induced by insulin was not assessed in this study Previous reports have shown that a 10 mg/kg dose of naloxone has no effect on insulin-induced hypoglycemia [19] Other reports have shown that higher doses of naloxone (20 mg/kg) blunts the insulin-induced hypoglycemia [7] Since we used a considerably lower dose of naloxone, it is unlikely that the results in this study can be explained by a reduction of the hypoglycemic activity of insulin following naloxone administration However, this possibility cannot be totally excluded as data from our earlier studies [24] showed that in a situation where blood glucose concentration was normal, insulin-induced feeding was insensitive to naltrexone treatment

An alternate explanation is that the opiate antagonists might reduce the insulin-induced feeding by inducing sickness in the rats as evidenced by their ability to induce conditioned taste aversion [6, 16, 21, 22] However, this possibility seems unlikely for two reasons First, naloxone failed to suppress the food intake when given just prior to the second hour of insulin-induced feeding, either in animals who had been injected with naloxone or in those who had not Secondly, most studies indicate that opiate antagonists are weak inducers of taste aversion [6, 21, 22] Only by using procedures designed to maximize sensitivity (two-stimulus tests) can the taste aversion property of naloxone and naltrexone be detected at 3 2 mg/kg dose [16]

Our earliest assumption was that insulin-induced feeding represented a non-endogenous opioid mediated mechanism, while feeding subsequent to 2-deoxy-D-glucose injection, food deprivation or spontaneous nocturnal feeding was mediated by endogenous opioids based on the sensitivity of these hyperphagias to naloxone [13,24] Recent data from our lab [3,4] and others [17,20] indicate that these naloxone reversible hyperphagias are not entirely equivalent, and may involve different opiate receptors [20] The present investigation offers further evidence that endogenous opioid involvement in feeding is far more complex than our earliest hypothesis, as insulin-induced feeding also involves a naloxone reversible component, as had been previously reported by others [7, 15, 20]

In summary, these data indicate the presence of two phases of insulin-induced feeding, the early phase being naloxone-sensitive while the later phase is naloxone-insensitive Additionally, the early, naloxone-sensitive phase begins with the presentation of food and not the actual administration of insulin

ACKNOWLEDGEMENTS

This study was supported in part by the American Cancer Society G 194 B, the Pardee Foundation, and a fellowship from The Purdue Research Foundation (E C C S) H U.B was supported as an APPE H A B Dunning Memorial Fellow

REFERENCES

1 Archer, S Historical perspective on the chemistry and development of naltrexone In *Naltrexone*, edited by R E Willette and G Barnett *NIDA Research Monograph* **28:** 3–10, 1980
2 Belluzzi, J D and L Stein. Do enkephalin systems mediate drive reduction? *Soc Neurosci Abstr* **4:** 405, 1978
3 Bryant, H U , M T Lowy, P V Malven, T D Steele and G K W Yim Effects of 2% sodium chloride imbibition on various opiate related hyperphagic conditions *Pharmacol Biochem Behav* **23:** 391–395, 1985
4 Bryant, H U , P V Malven and G K W Yim Differential inhibition by propranolol of feeding induced in rats by various stimuli *Pharmacol Biochem Behav* **21:** 651–654, 1984
5 Frenk, H and G H Rogers The suppressant effect of naloxone on food and water intake in the rat *Behav Neural Biol* **26:** 23–40, 1979
6 LeBlanc, A E and H Cappell Antagonism of morphine-induced aversive conditioning by naloxone *Pharmacol Biochem Behav* **3:** 185–188, 1975
7 Levine, A S and J E Morley Peptidergic control of insulin-induced feeding *Peptides* **2:** 261–264, 1981
8 Lowy, M T , R P Maickel and G K W Yim Naloxone reduction of stress related feeding *Life Sci* **26:** 2113–2118, 1980
9 Lowy, M T , C Starkey and G K W Yim Stereoselective effects of opiate agonists and antagonists on ingestive behavior in rats *Pharmacol Biochem Behav* **15:** 591–596, 1981
10 McDermott, L J and S P Grossman Circadian rhythms in ingestive behavior and responsiveness to glucoprivic and osmotic challenges in rats with rostral zona incerta lesions *Physiol Behav* **24:** 575–584, 1980
11 Melnyk, R B Insulin-induced feeding in hibernators *Behav Neural Biol* **32:** 70–78, 1981
12 Morley, J E and A S Levine Stress-induced eating is mediated through endogenous opiates *Science* **209:** 1259–1261, 1980
13 Morley, J E , A S Levine, G K W Yim and M T Lowy Opioid modulation of appetite *Neurosci Biobehav Rev* **7:** 281–305, 1983
14 Ngai, S. H , B A Berkowitz, J E Young, J Hempstead and S Spector Pharmacokinetics of naloxone in rats and in man: basis for its potency and short duration of action *Anesthesiology* **44:** 398–401, 1976
15 Ostrowski, N L , N Rowland, T. C Foley, T L Nelson and L D Reid Morphine antagonists and consummatory behaviors *Pharmacol Biochem Behav* **14:** 549–559, 1981
16 Pilcher, C W T and I P Stolerman Conditioned flavor aversions for assessing precipitated morphine abstinence in rats *Pharmacol Biochem Behav* **4:** 159–163, 1976
17 Przewlocki, R , W Lason, A M Konecka, A Herz and L D. Reid The opioid peptide dynorphin, circadian rhythms and starvation *Science* **219:** 71–73, 1983
18 Rowland, N Effect of insulin and 2-deoxy-D-glucose on feeding in hamsters and gerbils *Physiol Behav* **21:** 291–294, 1978.
19 Rowland, N and T J Bartness Naloxone suppresses insulin-induced food intake in novel and familiar environments, but does not affect hypoglycemia *Pharmacol Biochem Behav* **16:** 1001–1003, 1982
20 Simone, D A , R J Bodnar, E J Goldman and G W Pasternak Involvement of opioid receptor subtypes in rat feeding behavior *Life Sci* **36:** 829–833, 1985
21 Stolerman, I P , C W T Pilcher and G D D'Mello Stereospecific aversive property of narcotic antagonists in morphine-free rats *Life Sci* **22:** 1755–1762, 1978
22 Van Der Kooy, D and A G Phillips Temporal analysis of naloxone attenuation of morphine-induced taste aversion *Pharmacol Biochem Behav* **6:** 637–641, 1977
23 VanHouten, M , B I Posmer, B M Kopriwa and J R Brawer Insulin-binding sites in the rat brain In vivo localization to the circumventricular organs by quantitative autoradiography *Endocrinology* **105:** 666–673, 1979
24 Yim, G K W , M T Lowy, J M Davis, D R Lamb and P V Malven Opiate involvement in glucoprivic feeding In *The Neural Basis of Feeding and Reward*, edited by B G Hoebel and D Novin Brunswick Haer Institute, 1982, pp. 485–498