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Naloxone Suppresses Insulin-Induced Food Intake in Novel and Familiar Environments, but Does Not Affect Hypoglycemia

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ROWLAND, N. AND T. J. BARTNESS. *Naloxone suppresses insulin-induced food intake in novel and familiar environments, but does not affect hypoglycemia.* PHARMAC. BIOCHEM. BEHAV. 16(6) 1001–1003, 1982.—The opiate antagonist naloxone reduced the food intake induced in rats by acute injection of insulin. The suppression was most marked in the first hour after insulin injection. Insulin provoked less food intake when rats were tested in a novel environment compared with those tested in their home cage, but naloxone again significantly suppressed the intake in the first hour. Naloxone had no effect upon insulin-induced hypoglycemia.

Feeding Insulin Naloxone Novel environment Hypoglycemia

THERE is current disagreement concerning the effect of naloxone, an opioid antagonist, on insulin-induced food intake in rats. Lowy, Maickel and Yim [3] reported no effect of a range of doses of naloxone on 3 hr food intake after insulin. They interpret these and other [12] findings to mean that insulin feeding does not involve an endorphinergic mechanism. However, both Ostrowski *et al.* [6] and Levine and Morley [2] found that naloxone reduced insulin-induced feeding in the first hour after injection. The absolute food intakes in the latter [2] study were, however, extremely low, possibly because the rats were tested in a novel cage.

Levine and Morley [2] also reported a significant attenuation by naloxone of insulin-induced hypoglycemia. They took a single blood sample 2 hr after insulin (10 U/kg) and found glucose levels of 56 and 42 mg/dl in rats pretreated with naloxone (20 mg/kg) and saline, respectively. Such an attenuation of the hypoglycemia could, under some circumstances, result in decreased feeding (e.g. [8]). However, naloxone has no effect upon insulin-induced hypoglycemia in humans [9], so further clarification was necessary.

METHOD

Animals and Housing

Thirty-four male, laboratory-raised Long-Evans rats weighing 168–289 g were used in the behavioral study. They were divided into two groups at about 2 months of age. The home cage group were housed individually in hanging wire cages. The novel cage group were housed in groups of two in wire cages. Food (Purina chow pellets) and tap water were available ad lib. Overhead lights were on from 0800–2000.

Testing Conditions

On the designated test days, the home cage animals remained in their cage. The novel cage animals were placed individually in a novel polycarbonate cage (45×22×15 cm). All animals were weighed at about 1000 hr, and received injection of naloxone (10 mg/kg, SC; dose expressed as salt) or the saline vehicle. Food was removed from the home cage, or the animals were placed in the novel cage at this time. Fifteen min later, the animals received injection of regular insulin (10 U/kg, SC) or of saline. In each condition, six rats received saline-insulin, six received naloxone-insulin, and five received saline-saline. A weighed amount of food was presented immediately after the second injection (water was also available), and was replaced by fresh food at the end of the first, second and third hours. The hourly food intake, corrected for collected spillage, was measured. Ten days later, the tests were repeated, this time with the saline-insulin and the naloxone-insulin animals interchanged. Data from the two runs were similar, and have been combined.

Blood Glucose Changes

Nine male Long-Evans rats (220–360 g) were surgically implanted with indwelling catheters in the left jugular vein. After 3 days' recovery, the rats were injected with naloxone (10 mg/kg, SC) or saline, and 15 min later all received insulin (2 U/kg, SC). Blood samples (0.2 ml) were taken from the catheters (and 0.2 ml saline replaced) at the time of insulin injection (0 min), and after 10, 20, 30, 60, 120 and 180 min. The samples were collected in heparinized micro vials, cen-

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TABLE 1

EFFECTS OF NALOXONE (10 mg/kg) ON INSULIN (10 U/kg) INDUCED FOOD INTAKE AS A FUNCTION OF TEST ENVIRONMENT

Environment	Drug	Cumulative Food Intake (g) 1 hr	2 hr	3 hr
Home Cage	Naloxone	0.57 ± 0.12*	1.77 ± 0.24*	3.21 ± 0.37*
	Saline	1.70 ± 0.61	2.85 ± 0.64	4.30 ± 0.62
Novel Cage	Naloxone	0.24 ± 0.11*	0.43 ± 0.13*	2.38 ± 0.46†
	Saline	0.80 ± 0.02	2.05 ± 0.53	2.91 ± 0.51

Shown are Mean ± SE for N's 12.
*$p<0.01$, †$p<0.05$, less than corresponding saline value (Duncan New Multiple range test).

TABLE 2

EFFECT OF NALOXONE (10 mg/kg) OR SALINE PRETREATMENT ON BLOOD GLUCOSE LEVELS (mg/dl) AFTER INJECTION OF INSULIN IN FASTING RATS

	Time (min) after insulin 0	30	60	120	180
2 U/kg Insulin					
Saline (N=4)	119 ± 9	49 ± 2	40 ± 1	38 ± 6	57 ± 12*
Naloxone (N=5)	121 ± 11	53 ± 8	46 ± 6	43 ± 9	60 ± 11*
10 U/kg Insulin					
Saline (N=4)	106 ± 4	44 ± 3	29 ± 1	25 ± 2	19 ± 2*
Naloxone (N=5)	104 ± 2	38 ± 2	30 ± 2	19 ± 3*	21 ± 7*†

Shown are Mean ± S.E. blood glucose levels.
*One animal lethargic or comatose at time indicated.
†One animal died, and one animal excluded because injected with glucose at 120 min, so N=3 at this time.

trifuged, and the plasma assayed for glucose concentration using the glucose oxidase method (Sigma) on duplicate 30 μl samples. Four days later the experiment was repeated, using 10 U/kg insulin. On both days, food was not available during the test or for 2 hr (0830–1030) beforehand.

RESULTS

Table 1 shows the hourly cumulative food intakes of insulin-injected rats as a function of test environment for both naloxone and saline groups. Not shown are the intakes of rats receiving saline-saline treatment; these rats invariably ate much less than the saline-insulin groups (2.4 g/3 hr home cage; 2.0 g/3 hr novel cage), indicating that insulin did indeed have a stimulatory effect upon food intake.

A 3-way ANOVA (Environment × Drug × Time) was performed on the data shown in Table 1. Significant main effects of drug regime: $F(1,22)=13.36$, $p<0.001$, environment: $F(1,22)=5.50$, $p<0.05$, and time: $F(2,44)=92.91$, $p<0.001$ were obtained. None of the 2-way or 3-way interactions approached significance. Duncan's new Multiple Range post hoc tests [1] revealed greater food intake for saline-insulin than naloxone-insulin groups ($p<0.01$), and a greater food intake in the home cage than in the novel cage ($p<0.05$).

Table 2 shows the blood glucose results; since glucose was little reduced after 10 min, and the 20 min values were similar to those obtained at 30 min, we give only the data for the 30 min, 1, 2, and 3 hr samples. Naloxone had no effect on the time 0 blood glucose level. There were no differences between the groups at any time point after either dose of insulin. At the higher dose, one of the naloxone treated rats was convulsing after 2 hr, and was withdrawn from the experiment for glucose injection, and a second animal died before the 3 hr reading. The 3 hr reading is thus biased upward in the naloxone group. The means of the individual nadir values were 40 mg/dl (saline/2 U group, nadirs after 1–2 hr); 35 mg/dl (naloxone/2 U group, 1–2 hr); 19 mg/dl (saline/10 U group, 3 hr); 16 mg/dl (naloxone/10 U group, 2–3 hr). These indicate greater hypoglycemia after the higher dose of insulin, but that naloxone had no significant effect. If anything, naloxone exacerbated the hypoglycemia.

DISCUSSION

The present home cage data are virtually indistinguishable from those we obtained previously [6]. We find much reduced eating in the novel cage situation, and our data are quite similar to those reported by Levine and Morley [2]. In both cases, the naloxone effect is not evident after 3 hr, and can account for the reported failure [3] to observe inhibition

of insulin-induced feeding. We should emphasize, however, that under some circumstances the first hour intake after insulin might be so low that no further inhibitory effect might be observable (floor effect). Unless a substantial hour 1 intake is obtained, then a naloxone suppression might be missed. We should also emphasize that our "novel cage" rats were additionally isolated from their normal cage-mate for the duration of the test. This deliberate strategy was to maximize the novelty of the testing situation.

There is a concensus (e.g., [3,6]) that food intake elicited by another glucoprivic agent, 2-deoxy-D-glucose, is suppressed by naloxone for as long as 3 hr. One possible rationalization of these data is that 2DG-induced feeding is more easily disrupted than that induced by insulin, a finding which has precedent in some brain-damaged preparations (e.g., [11]). Alternatively, the different effects of naloxone might reflect different sites of action of the two glucoprivic stressors [8,10]. A third possibility, suggested by Yim and his colleagues [12], is that insulin-induced feeding may be opiate independent, while 2DG feeding involves opioids. This is supported by their behavioral study [3] and an abstract report [12] of elevated plasma β-endorphin-like activity after 2-deoxy-D-glucose, but not after insulin treatment. If the observation that naloxone suppresses a behavior is indeed evidence for opioid involvement, then our present data do not support Yim's proposition [12], but instead argue in favor of a naloxone-sensitive component, at least in the first hour following insulin treatment.

In order to make a rigorous comparison of the two glucoprivic stimuli it may be important to equate them for the amount of induced food intake. This was effectively accomplished in our own [6], but not in Lowy *et al.*'s [3] study. We have recently found that water intake provoked by various dipsogens is differentially affected by naloxone [7], and the importance of equating intakes was raised in that study.

We found no evidence for an altered hypoglycemic response to insulin after naloxone treatment at a low dose which might involve principally hepatic mechanisms in feeding [8], or after a high dose which might additionally involve cerebral receptors [10]. The reasons for the discrepancy with Levine and Morley's [2] data are unclear, but our result does agree with negative findings in humans [9]. Other experiments have, however, indicated that opiate receptors may be modulated by blood glucose level [5], and that naloxone may modify carbohydrate metabolism in fasted man [4]. Further physiological and behavioral characterization is needed.

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