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Low Doses of Naloxone and MIF-1 Peptides Increase Fluid Consumption in Rats[1]

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OLSON, R. D., R. C. FERNANDEZ, A. J. KASTIN, G. A. OLSON, S. W. DELATTE, T. K. VON ALMEN, D. G. ERICKSON, D. C. HASTINGS AND D. H. COY. *Low doses of naloxone and MIF-1 peptides increase fluid consumption in rats*. PHARMAC. BIOCHEM. BEHAV. 15(6)921–924, 1981.—Three studies were done with albino rats to determine the effects of low doses of opiate antagonists on fluid intake. In Experiment 1, male rats were deprived of water for 12 hr and then randomly injected IP with 0.0, 0.01, 0.1, 1.0, or 10.0 mg/kg of naloxone. Ten min later they were given free access to a 20% sucrose solution and consumption was measured for the next 30 and 60 min on 2 consecutive days. Only animals injected with 1.0 or 10.0 mg/kg drank significantly less than controls. The other doses were not reliably different from controls but on Day 1 animals injected with 0.01 mg/kg of naloxone drank slightly more than controls. Experiment 2 followed the same procedure with lower doses of 0.0, 0.001, 0.01, and 0.1 mg/kg of naloxone. Although the effect of 0.1 mg/kg of naloxone was again not significant, this time animals injected with 0.001 and 0.01 mg/kg of naloxone consumed reliably more fluid than controls. Experiment 3 extended the findings to a peptide with opiate antagonistic properties and its analogs. Male and female rats were randomly assigned to receive an IP injection of 0.0, 0.01, 0.1, or 1.0 mg/kg of naloxone, MIF-1 (Pro-Leu-Gly-NH_2), the pGlu analog (pGlu-Leu-Gly-NH_2), or Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2). Measurement of intake occurred every 30 min or 600 min. Consumption was significantly increased. Rats injected with MIF-1 drank the most, followed in order by those injected with the pGlu analog, naloxone, Tyr-MIF-1, and controls. Dose was also reliable in a dose-dependent fashion, with animals receiving the lowest dose of 0.01 mg/kg drinking the most. None of the groups drank less than the controls. Sex was also significant in interactions with substance and dose. The results suggest that in some situations low doses of opiate antagonists may facilitate fluid consumption even though high doses are known to suppress the same behavior. The data also support the role of MIF-1 and MIF-1 analogs as opiate antagonists.

Naloxone MIF-1 MIF-1 analogs Tyr-MIF-1 Fluid intake Sex differences Rats

THERE is a growing body of evidence implicating the role of endogenous opiate systems in modulating food and water consumption. Starting with the report that genetically obese mice have increased levels of endogenous opiates [8], other studies have focused on the ability of opiate antagonists such as naloxone to suppress eating and drinking [1] and have usually reported that suppression occurs in a dose-dependent fashion [7], even when using moderately low doses [9]. It has also been reported that fluid intake is much more sensitive to modulation than food [7], and that attractive fluids such as sucrose solutions are even more sensitive [9]. Although the mechanism through which opiate agonists and antagonists exert their effect is not known, we have previously demonstrated that neither the ventromedial hypothalamus [7] nor the vagus nerve [4] is essential.

It has also been demonstrated with fluid-intake paradigms that MIF-1 (Pro-Leu-Gly-NH_2) has effects comparable to those associated with naloxone [9], and may function as an endogenous opiate antagonist in some situations [6]. Evidence for this role as an antagonist is growing and is based on similar results in tests comparing naloxone and MIF-1 such as their effects on tonic immobility in lizards [2], morphine dependence [10,11], and tail-flick analgesia [6]. Only the work on morphine dependence has been equivocal [10,11].

Although we previously demonstrated that MIF-1 and naloxone acted in a comparable fashion and generally suppressed fluid intake, rats injected with 0.1 mg/kg of naloxone drank much more than those injected with higher doses or even similar doses of MIF-1 [9]. Since studies of modulation of eating and drinking by naloxone typically are done with moderate to high doses [1,7], it was not clear if our finding was an anomaly or suggestive of a bi-directional effect for naloxone.

Thus, the purpose of the present study was to evaluate

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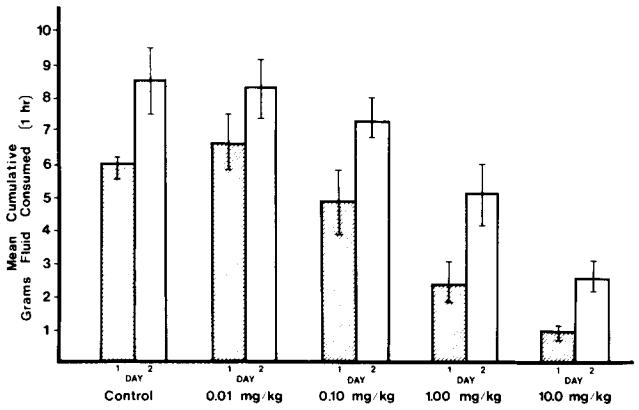

FIG. 1. Mean (±S.E.M.) cumulative g of fluid consumed over 1 hr for each dosage level on consecutive days for Experiment 1.

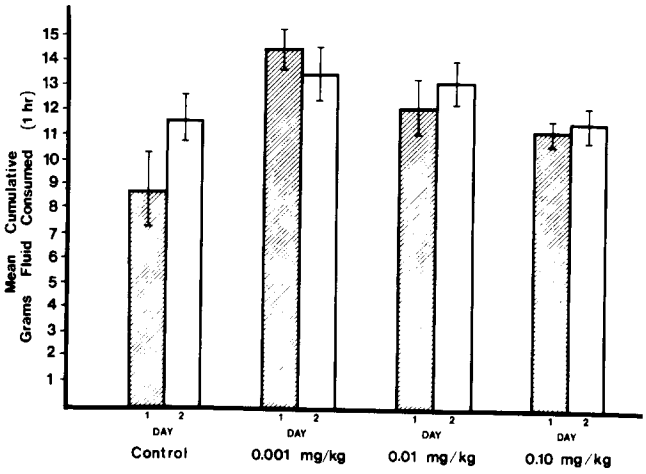

FIG. 2. Mean (±S.E.M.) cumulative g of fluid consumed over 1 hr for each dosage level on consecutive days for Experiment 2.

the effects of very low doses of opiate antagonists in a standard fluid-intake paradigm. Further, the effects of sex were examined because of previous work suggesting interactions.

EXPERIMENT 1

To evaluate the effects of small amounts of naloxone on fluid intake, it was decided to compare amounts ranging from a moderate amount known to typically suppress intake (10.0 mg/kg) to a low amount not usually studied (0.01 mg/kg). The effects of repeated injections were also examined to see if any signs of tolerance developed to the opiate antagonist.

METHOD

Animals

Rats (n=30) derived from the Sprague-Dawley strain were obtained from King Laboratories (Oregon, WI) and housed individually in a temperature controlled colony (22–24°C) with a 12 hr light/dark cycle (light onset 8:00 a.m.) throughout the experiment. The experimentally naive male rats averaged 210.4 g at the start of testing.

Drugs

All animals received either 0.0, 0.01, 0.1, 1.0, or 10.0 mg/kg of naloxone dissolved in a vehicle consisting of 0.9% saline made with acetic acid to 0.01 M. Injections were given in a volume proportional to weight of 1.0 ml/kg; all injections were administered IP.

Procedure

Animals were housed individually in the colony with free access to food and water for 5 days before the experiment. At 8:00 p.m. the night before testing, all water was removed from the cages. Starting at 8:00 a.m. the next morning, the animals received the appropriate injection, were returned to their home cages, and 10 min later were given free access to a bottle of 20% sucrose solution. Recordings of fluid intake were made after 30 and 60 min on two consecutive days.

RESULTS

The results for Experiment 1 are presented in Fig 1. A mixed analysis of variance (dose by days by min) was performed on the amount of fluid consumed. The main effect for dose was highly reliable, $F(4,25)=21.968$, $p<0.01$, with Scheffe's test showing significant suppression of intake in several groups, all in a dose-dependent fashion. Rats injected with 10.0 mg/kg drank reliably less than all other groups and rats injected with 1.0 mg/kg drank reliably less than all groups receiving a smaller dose. None of the other comparisons between 0.1, 0.01, and 0.0 were significant, but on Day 1, animals injected with 0.01 mg/kg drank slightly more liquid than controls.

The main effect for days was reliable, $F(1.25)=24.818$, $p<0.01$, with more consumption taking place on Day 2. The main effect for min was also significant, $F(1,25)=18.669$, $p<0.01$, with greater intake occurring during the first 30 min. No other reliable effects were obtained.

EXPERIMENT 2

The suppression in drinking associated with moderate to high doses of naloxone obtained in Experiment 1 indicated the efficacy of the paradigm. The lack of significant increases in consumption, however, suggested that the doses were not low enough or that the bi-directional action did not exist. Accordingly, the dose was lowered to 0.001 mg/kg of naloxone and the moderate to high doses of naloxone were omitted. All other factors from the first experiment remained constant.

METHOD

Animals

Rats (n=20) were obtained from King Laboratories and maintained exactly as described in Experiment 1. The average weight of the experimentally naive male rats was 228.1 g at the start of testing.

Drugs

All animals received either 0.0, 0.001, 0.01, or 0.1 mg/kg of naloxone dissolved in the vehicle of acidified saline. Injections were given IP in a volume of 1.0 ml/kg.

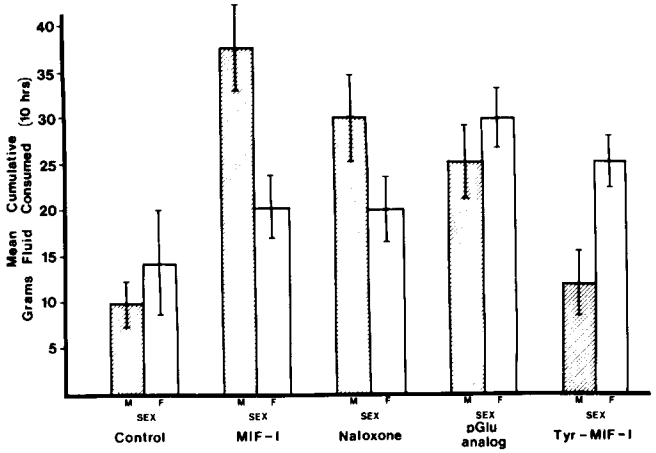

FIG. 3. Mean (±S.E.M.) cumulative g of fluid consumed over 10 hr in all treatment groups for males and females for Experiment 3.

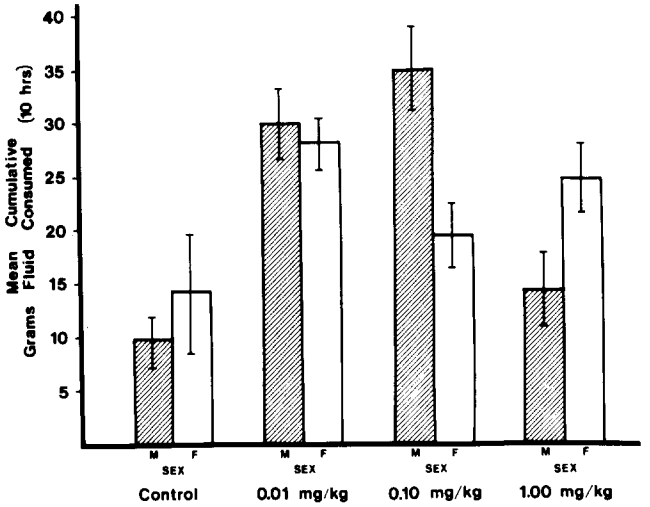

FIG. 4. Mean (±S.E.M.) cumulative g of fluid consumed over 10 hr for each dosage level across combined treatment groups for males and females in Experiment 3.

Procedure

The procedure was identical to that used in Experiment 1.

RESULTS

Figure 2 presents the data for this study. A mixed analysis of variance (dose by days by min) was again performed on the amount of fluid consumed. The main effect for dose was significant, $F(3,16)=4.994, p<0.05$, with Scheffe's test showing significant increases in consumption for animals injected with 0.001 mg/kg relative to animals injected with either 0.1 or 0.0 mg/kg of naloxone, and also for rats injected with 0.01 mg/kg of naloxone relative to controls. No other comparisons were reliable.

The main effect for min was again significant, $F(1,16)=103.302, p<0.01$, with more consumption occurring during the first 30 min. No other results were reliable.

EXPERIMENT 3

The significant increase in fluid intake by animals injected with 0.001 and 0.01 mg/kg of naloxone suggests the notion of bi-directional effects associated with opiate antagonists but was somewhat surprising since animals injected with 0.01 mg/kg of naloxone in Experiment 1 did not perform significantly differently from controls. Accordingly, we decided to test the effects of low doses of naloxone again, and also to add MIF-1 and other MIF-1 analogs to evaluate their role as possible opiate antagonists. Sex was also added as a variable.

METHOD

Animals

Rats (n=104) were obtained from Simonsen Laboratories (Gilroy, CA) and maintained exactly as described in Experiment 1. The average weight at the start of testing of the 52 females was 188.6 g and the average weight of the males was 288.9 g.

Drugs

The following substances were used: naloxone, MIF-1 (Pro-Leu-Gly-NH_2), the pGlu analog (pGlu-Leu-Gly-NH_2), and [Tyr^1]-MIF-1 (Tyr-Pro-Leu-Gly-NH_2). All compounds were dissolved in the vehicle previously mentioned. Doses of 0.01, 0.1, and 1.0 mg/kg of each substance were prepared and administered IP in a volume of 1.0 ml/kg. In all 3 experiments in this paper, substances and doses were prepared and coded by persons other than those doing the injections and data collection, and the codes were not revealed until after analysis of the data had been completed.

Procedure

All testing was done on 5 consecutive days; sex and order of administration of substance and dose were counterbalanced. Each animal was tested only once for a period of 10 hr with measurements being made every 30 min. All other aspects of the procedure were identical to those previously described.

RESULTS

A mixed analysis of variance (sex by substance by dose by min) was performed on the data. The main effect for substance was significant, $F(3,72)=6.767$, $p<0.01$, and Scheffe's test indicated that all the possible comparisons between naloxone, MIF-1 Tyr-MIF-1, and the pGlu analog were reliable except between MIF-1 and the pGlu analog (Fig. 3). Thus, animals injected with MIF-1 drank the most, followed by those injected with the pGlu analog, then naloxone, and Tyr-MIF-1. The single control group was not included in the overall analysis but was compared to each dose of each substance individually by Dunnett's test. This analysis showed all groups drank significantly more than controls except the Tyr-MIF-1 1.0 mg/kg and naloxone 1.0 mg/kg. These groups drank more than controls but were not reliably different.

The main effect for dose was also significant, $F(2,72)=8.852$, $p<0.01$, with all groups responding in a dose-dependent fashion. Scheffe's test performed on the various dose comparisons revealed that all levels were reliably different from each other, with rats injected with 0.01 mg/kg drinking the most, followed by those injected with 0.1 mg/kg and then those injected with 1.0 mg/kg (Fig. 4). The other between-groups main effects, sex, was not significant.

Two interactions were significant. Sex by substance, $F(3,72)=9.718$, $p<0.01$, was significant and was further analyzed using the F test for simple effects. This test indicated that males drank more than females after injections of MIF-1 or naloxone, about the same after injections of the pGlu analog, and less after injections of Tyr-MIF-1. Sex by dose was also significant, $F(2,72)=17.570$, $p<0.01$, and was again evaluated with the F test for simple effects. At 0.01 mg/kg and 0.1 mg/kg males drank reliably more than females, but consumed significantly less than females after injections of 1.0 mg/kg. In all tests, males drank the most after a 0.1 mg/kg injection while females drank the least after the identical dose.

Time was also significant, $F(19,1368)=2.599$, $p<0.01$, with slightly more drinking in the early part of the study. No other reliable results were obtained.

DISCUSSION

The results of these experiments vary considerably from previous studies investigating the effects of naloxone on fluid consumption because of the facilitation measured in animals receiving low doses of naloxone, MIF-1, and other MIF-1 compounds. Extreme caution must be used in the interpretation of these data, however, because of the inconsistent nature of the effect. The series of three studies reported in this paper were conducted under highly similar conditions and still did not yield total reliability. It was clear, however, that lower doses of naloxone than those usually evaluated consistently produced increased consumption or consumption no different from that observed in controls; in no case did the lowest doses (0.001 and 0.01 mg/kg) produce suppression.

Once again evidence has been obtained suggesting that MIF-1 has effects comparable to those of naloxone. The pGlu analog and Tyr-MIF-1 were also effective and similar to naloxone in terms of action. The pGlu analog has been reported to be more potent than MIF-1 in reversing the tremors induced by oxotremorine [3], and was also very potent in this study. Tyr-MIF-1, a naturally occurring MIF-1 tetrapeptide, recently determined by radioimmunoassay to be present in the pineal gland [5], may be considered the weakest of the substances in facilitating consumption or the strongest of the peptides in suppressing intake, suppression being the traditional result of antagonist administration.

Several sex differences were obtained in agreement with results from a wide variety of paradigms that suggest a differential effect for the peptide as a function of the organism's sex [2]. In the current study, males drank more after being injected with MIF-1, followed in order by naloxone, the pGlu analog, and Tyr-MIF-1. Females drank the most after injections of the pGlu analog, then Tyr-MIF-1, MIF-1, and naloxone. The sexes also reacted differentially to doses, with males drinking the most and females the least after a dose of 0.1 mg/kg. The nature of action for these differences is not clear.

In Experiment 1 there was an increased consumption by all groups on Day 2 that could be due to increased acceptance of the novel 20% sucrose solution. It might also be due in part to the development of tolerance to the naloxone. It is possible that large doses of naloxone or other opiate antagonists administered chronically would ultimately generate tolerance and/or alter standard physiological reactions to facilitate intake rather than suppress it.

The results suggest that in some cases low doses of an opiate antagonist will increase fluid intake. They are also consistent with the idea that MIF-1 and its analogs might act as endogenous opiate antagonists in some situations.

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