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Continuous Infusion of Naloxone: Effects on Behavior and Oxygen Consumption[1]

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MALIN, D. H., J. G. LEAVELL, K. FREEMAN, W. C. KINZLER AND M. A. REAGAN. *Continuous infusion of naloxone: Effects on behavior and oxygen consumption.* PHARMACOL BIOCHEM BEHAV 22(5) 791–795, 1985.—Twenty-eight hours of endorphin receptor blockade by subcutaneous naloxone infusion produced behavioral and respiratory symptoms resembling opiate abstinence syndrome. Rats were implanted subcutaneously with two Alzet osmotic minipumps delivering 0.7 mg/kg per hour naloxone or with two control minipumps containing distilled water only. They were observed for 10 minutes under blind conditions at 16 and 28 hours post-implantation. The naloxone-infused rats showed significantly more wet dog shakes, abdominal writhes and overall abstinence-like symptoms than did the control rats. These symptoms decreased after 28 hours despite continued naloxone infusion. Acute administration of naloxone failed to produce abstinence-like symptoms, even when combined with the trauma of carrying two implanted water-filled minipumps for 28 hours. In another experiment, naloxone-infused rats showed a highly significant 53.4% elevation of O_2 consumption over water-infused control rats in a pure O_2 atmosphere at 28 hours after implantation. This difference disappeared at 48 hours post-implantation. In contrast to the effect of naloxone infusion, acute administration of three different doses of naloxone failed to significantly increase O_2 consumption.

Naloxone Opiate antagonists Endorphins Opiate abstinence syndrome Respiration

ENDORPHINS produce many of the same physiological and behavioral effects as opiate narcotic drugs, by acting on many of the same receptors. When these receptors are blocked in an opiate-dependent organism, a characteristic opiate abstinence syndrome results. Several recent studies suggest the existence of an ongoing daily rhythm of endorphinergic stimulation. Daily rhythms of dynorphin-like immunoreactivity [14] and naloxone-reversible fluctuations in pain sensitivity [5] have been reported. This raises the question of whether depriving an opiate-naive animal of its normal, ongoing endorphin-receptor stimulation would result in symptoms resembling opiate abstinence syndrome. Naloxone selectively and stereospecifically blocks endorphin receptors. Acute naloxone administration, even at high doses, generally fails to produce an abstinence-like syndrome. However, twice daily injections of 0.6 mg/kg naloxone (for seven days in one experiment and ten days in another) did result in many abstinence-like behavioral symptoms [12].

Since naloxone is a short-acting drug, the endorphin-receptor blockade resulting from twice daily injections is far from continuous. If prolonged absence of endorphin-receptor stimulation is responsible for the abstinence-like symptoms, continuous exposure to naloxone should produce such symptoms more quickly. In order to produce such continuous exposure, our laboratory used surgically implanted Alzet model 2001 osmotic minipumps, which have been shown to deliver 1 μl per hour for at least seven days [18,19]. Several experiments were performed to determine whether naloxone subcutaneously infused from these pumps would produce the behavioral symptoms and respiratory hyperactivity typical of opiate abstinence syndrome.

EXPERIMENT 1

Method

Subjects were 12 male Sprague-Dawley rats weighing an average of 141 g with a range of 130–150 g. Throughout the experiment, rats were housed in groups of three, maintained on a twelve hour light and dark cycle (lights on from 10 a.m. to 10 p.m.), and on ad lib food and water.

Two Alzet 2001 osmotic minipumps were implanted subcutaneously in the shoulder region of each rat, under light ether anesthesia. Each cylindrical pump was 3.0 cm long, 0.7 cm in diameter, and weighed 1.34 g when filled.

The rats were randomly divided into two groups. Group 1 (n=6) received two pumps containing 0.48 ml of 50 mg/ml naloxone in distilled water. Thus, each rat received an average of 0.7 mg/kg naloxone per hour on a continuous basis (with a range from 0.67 to 0.77 mg/kg per hour). Group 2 was implanted with two pumps containing distilled water only.

Behavioral observations consisted of ten-minute tests in a 45×45×30 cm clear plastic chamber with an open top and a wire mesh floor. At 16 and 28 hours post-implantation, the

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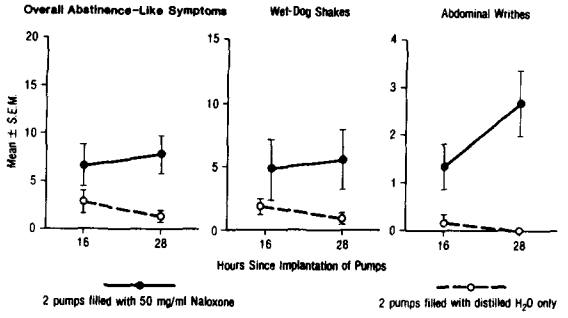

FIG. 1. Wet-dog shakes, abdominal writhes and overall abstinence-like symptoms (mean±SEM) in six rats implanted subcutaneously with two Alzet 2001 osmotic minipumps filled with 50 mg/ml naloxone and in six rats implanted with two minipumps filled with distilled water only. Rats were observed for 10 min at 16 and 28 hours post implantation.

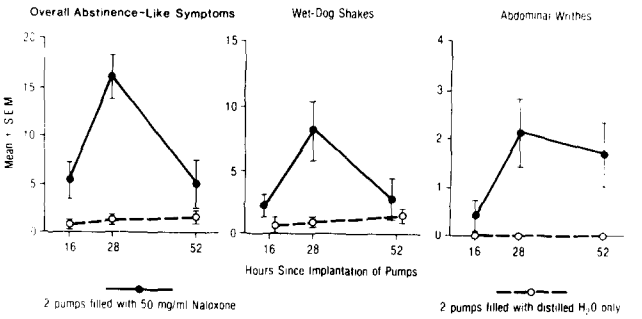

FIG. 2. Wet-dog shakes, abdominal writhes and overall abstinence-like symptoms (mean±SEM) in five rats implanted subcutaneously with two Alzet 2001 osmotic minipumps filled with 50 mg/ml naloxone and in five rats implanted with two minipumps filled with distilled water only. Rats were observed for 10 min at 16, 28, and 52 hours post implantation.

rats were scored for behavioral abstinence symptoms according to the criteria of Gianutsos, Drawbaugh, Hynes and Lal [6]. Since there is a four-hour delay until drug infusion reaches its full rate, this constituted 12 and 24 hours of full-rate infusion. A standard tally sheet was used to record the frequency of abdominal writhes, wet-dog body shakes and head shakes, ptosis, teeth grinding, dyspnea and hind-foot scratching. All solutions and animals were coded, and observations were performed under "blind" conditions by a single observer. As a check on the reproducibility of the scoring methods, inter-observer reliability of these procedures was previously established (r=0.903). All rats were weighed immediately prior to implantation, and at 28 hours post-implantation.

Results

An analysis was made of overall abstinence-like symptoms (total frequencies for each animal across all symptom categories). In addition, separate analysis were made of wet-dog shakes and abdominal writhes, because they have proved to be sensitive indicators of opaite abstinence syndrome [6].

As Fig. 1 indicates, naloxone-infused rats had significantly more body shakes, writhes, and overall abstinence-like symptoms as compared with water-infused controls. A 2×2 analysis of variance (ANOVA) of overall symptoms, with one factor having 2 repeated measures, revealed a significant drug effect: naloxone vs. water, $F(1,10)=5.94$, $p<0.05$. The time effect was not significant: 16 vs. 28 hours, $F(1,10)=0.0$, NS; nor was the interaction effect: drug × time, $F(1,10)=0.21$, NS.

A separate ANOVA for body shakes revealed a significant drug effect: naloxone vs. water, $F(1,10)=12.74$, $p<0.01$. The time effect was not significant, $F(1,10)=0.0$, NS; nor was the interaction effect: drug × time, $F(1,10)=0.21$, NS.

A separate ANOVA for abdominal writhes revealed a significant drug effect, $F(1,10)=5.98$, $p<0.05$. Once again, neither the time effect, $F(1,10)=0.53$, NS, nor the interaction effect, $F(1,10)=2.11$, NS, was significant.

The water-infused control rats gained 3.0±0.1 g (mean±SEM) during the 28 hours following pump implantation, while the naloxone-infused rats gained only 2.3±0.1 g. This difference was significant, $t(10)=3.66$, $p<0.005$.

EXPERIMENT 2

Experiment 2 was performed to test the repeatability of Experiment 1 and to ascertain whether the effect of naloxone infusion reached a peak within two days.

Method

Procedures were the same as in Experiment 1, with the following exceptions: subjects were 10 rats, randomly divided into an experimental group (n=5), each receiving two naloxone-filled pumps; and a control group (n=5), each receiving two distilled water pumps. Animals were observed under "blind" conditions for abstinence-like symptoms at 16, 28, and 52 hours post-implantation.

Results

Figure 2 illustrates that, once again, naloxone-infused rats had more wet-dog shakes, abdominal writhes and overall abstinence-like symptoms than did water-infused control rats. In each case, the effect of naloxone infusion reached a peak at 28 hours post-implantation.

Total abstinence-like symptoms were subjected to 2×3 ANOVA with one factor having three repeated measures. The drug effect was highly significant: naloxone vs. water, $F(1,14)=22.44$, $p<0.001$, as was the time effect, $F(2,28)=9.33$, $p<0.01$, and the interaction effect: drug × time, $F(2,28)=9.40$, $p<0.01$. In view of the significant interaction, an analysis of simple main effects was performed to investigate the significance of the drug effect at various times of testing. At 16 hours, the difference between naloxone and control groups approached significance, $F(1,42)=3.80$, $0.05, p<0.10$. At 28 hours, the difference was highly significant, $F(1,42)=40.89$, $p<0.001$. At 52 hours, the difference was no longer significant, $F(1,42)=2.14$, NS.

A separate ANOVA for wet-dog shakes revealed a significant drug effect, $F(1,14)=4.97$, $p<0.05$, as well as time effect, $F(2,28)=6.82$, $p<0.01$, and interaction effect: drug × time, $F(2,28)=7.46$, $p<0.01$.

A separate ANOVA of abdominal writhes revealed a significant drug effect, $F(1,14)=6.30$, $p<0.05$. However, neither the time effect, $F(2,28)=0.84$, NS, nor the interaction effect: drug × time, $F(2,28)=0.84$, NS, was significant.

EXPERIMENT 3

This experiment was performed to test whether chronic [12] or continuous as opposed to acute endorphin-receptor blockade is necessary to produce abstinence-like signs. Might a single high dose naloxone injection produce these same effects, if combined with the mechanical irritation of carrying two minipumps for a day?

Method

Procedures were the same as in Experiment 1 with the following exceptions. Group 1 (n=6) was implanted subcutaneously with two Alzet 2001 osmotic minipumps filled with distilled water only. Group 2 (n=6) received no surgical treatment.

Twenty-eight hours after implantation, all rats were injected subcutaneously with 2.5 ml/kg distilled water. Twenty minutes following injection, rats were observed for ten minutes and scored for abstinence-like symptoms. The same rats were then injected with 2.5 ml/kg distilled water containing 3 mg/kg naloxone. Twenty minutes later, each rat was again observed for ten minutes.

Results

The pump-carrying rats demonstrated only 0.8±0.3 (mean±SEM) overall symptoms following water injection, while the non-pump-carrying rats demonstrated only 0.2±0.2 symptoms. Subsequent naloxone injection failed to markedly increase symptoms in either group. After naloxone injection, the pump-carrying rats demonstrated only 0.7±0.3 overall symptoms, while the non-pump-carrying rats demonstrated 0.3±0.2 symptoms. A 2×2 analysis of variance with one factor having 2 repeated measures, revealed no significant effect of the presence or absence of pumps, $F(1,10)=4.29$, NS. There was no significant drug effect (water versus naloxone), $F(1,10)=0.0$, NS, nor interaction effect, $F(1,10)=0.34$, NS.

EXPERIMENT 4

In Experiments 1 and 2, naloxone-infused rats exhibited behaviors resembling those commonly exhibited by rats undergoing opiate abstinence syndrome. However, one might speculate that these behavioral similarities need not reflect similar underlying physiological states. Narcotic abstinence produces such characteristic physiological changes as increased respiratory activity and O_2 consumption. This experiment tested for such changes in naloxone-infused animals by placing them in a custom-designed apparatus for measuring O_2 consumption in the unrestrained rat.

Method

Measurement of oxygen consumption was modified from the soda-lime method of Watts and Gourley [24]. The modifications permitted the use of an unrestrained animal, thus avoiding immobilization stress, which might distort respiratory activity. The rat was placed in a 2000 ml reaction vessel, the bottom of which was covered with 6–12 mesh soda-lime with saturation indicator (Fischer Scientific). A grid floor held the rat above the soda-lime. The removable lid of the reaction vessel was fitted with an inlet valve leading to an O_2 canister, and with two outlet valves. This allowed the chamber to be purged with a pure O_2 atmosphere, a standard procedure to minimize respiratory effects of transient CO_2 buildup [4,24]. Another outlet was connected through 0.25 inch plastic tubing in a "J" arrangement to a 5 ml microburette, filled with water which was allowed to seek equilibrium on both sides of the "J."

As the rat within the vessel breathed, it produced CO_2 which was absorbed by the soda-lime, thus reducing the gas volume in the cylinder and causing the liquid to flow toward the reaction vessel. Thus the fall of the liquid level in the burette measured the O_2 volume consumed by the animal.

The subjects were 12 male Sprague-Dawley rats, weighing 218–231 g, an optimal size for the apparatus. Each rat was habituated to the apparatus by placing it within the chamber, purging the chamber, and letting the rat remain for five minutes. This habituation procedure was repeated each day during the four days prior to surgical implantation. Each rat was subsequently implanted in the scapula region with two Alzet 2001 osmotic minipumps. Rats were randomly divided into two groups. Group 1 (n=6) received pumps filled with 0.48 ml of 50 mg/ml naloxone in distilled water. Thus each rat received approximately 0.45 mg/kg/hour naloxone on a continuous basis. Group 2 (n=6) received pumps filled with distilled water only.

Each rat was tested for baseline O_2 consumption during five minutes before pump implantation and was retested at 27 and 48 hours after implantation. The rat's oxygen consumption during a post-implantation test was expressed as a percentage increase over its baseline rate.

Results

Pre-implantation O_2 consumption was 0.53±0.02 ml for the group that received water-filled pumps and 0.54±0.03 ml for the group that received naloxone-filled pumps. This difference was not significant, $t(10)=0.09$, NS. As Fig. 3 illustrates, the naloxone-infused rats increased their O_2 consumption by an average of 53.4% over baseline at 27 hours post-implantation. However, by 48 hours post-implantation, the naloxone-infused rats were slightly below their baseline O_2 consumption. The water-infused rats remained close to their baseline O_2 consumption throughout. A 2×2 ANOVA with one factor having 2 repeated measures revealed a significant drug effect: naloxone vs. water, $F(1,10)=10.62$, $p<0.01$; a significant time effect: 27 vs. 48 hours, $F(1,10)=18.58$, $p<0.01$; and a significant interaction effect: $F(1,10)=31.31$, $p<0.001$.

Analysis of simple main effects revealed a highly significant difference between naloxone-infused and H_2O-infused animals at 27 hours post-implantation, $F(1,20)=39.80$, $p<0.001$, but not at 48 hours, $F(1,20)=3.26$, NS.

EXPERIMENT 5

This experiment attempted to assess whether prolonged endorphin-receptor blockade was necessary to produce increased O_2 consumption, or whether the same effect could be produced by acute naloxone injection, at any of a wide range of doses.

Method

Procedures were the same as in Experiment 4 with the following exceptions. Thirty rats, pre-habituated to the O_2 apparatus, were tested for baseline O_2 consumption during a five minute interval. Five rats received 0.7 mg/kg naloxone subcutaneously. Five rats received 2.5 mg/kg naloxone. Five rats received 11.4 mg/kg naloxone. Fifteen rats received distilled water alone. The 0.7 mg/kg dose approximated the amount of drug received in an hour of continuous infusion in

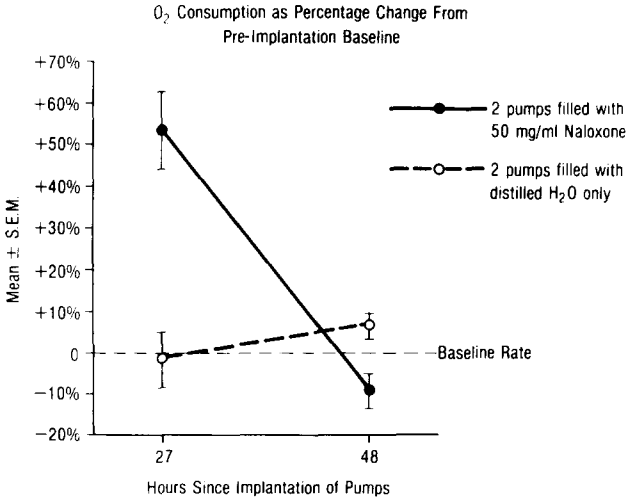

FIG. 3. Percentage change (mean±SEM) from pre-implantation baseline in oxygen consumed during a five min test. Six rats were implanted subcutaneously with 2 Alzet 2001 minipumps filled with 50 mg/ml naloxone. Six rats were implanted with 2 pumps filled with distilled water only. A baseline test was performed immediately before pump implantation and each rat was retested at 27 and 48 hours post-implantation. Baseline oxygen consumption was 0.54±0.03 ml for the rats subsequently implanted with naloxone pumps and 0.53±0.02 ml for the rats subsequently implanted with water pumps.

Experiment 1, while the 11.4 mg/kg dose approximated the cumulative amount of naloxone received after a day of full-rate infusion (a time of peak abstinence-like symptoms). The 2.5 mg/kg dose is in the range commonly employed to inhibit endogenous opioid systems [5]. Oxygen consumption was measured again for five minutes, beginning fifteen minutes after injection. Each animal's score was expressed as a percentage change from its pre-injection baseline.

Results

The results are summarized in Table 1. The baseline O_2 consumption of the four groups did not differ significantly according to one-way ANOVA, $F(3,26)=2.17$, NS. None of the three injection doses of naloxone produced a major increase in O_2 consumption such as that produced by continuous naloxone infusion in Experiment 4.

One-way ANOVA of percentage changes from baseline revealed a significant naloxone dose effect, $F(3,26)=11.2$, $p<0.01$. Dunnett's tD test for multiple comparisons with a single control group [8] showed that the 0.7 and 2.5 mg/kg doses each produced a significantly greater decrease in O_2 consumed than did injection of distilled water alone, while the 11.4 mg/kg dose produced no significant effect.

DISCUSSION

Continuous naloxone blockade of the rat's normal, ongoing endorphin-receptor stimulation resulted in a pattern of behavioral distress symptoms coupled with respiratory hyperactivity and retarded weight gain. This resembled the pattern commonly observed in opiate abstinence syndrome. The symptoms developed in one day, as contrasted with the seven days required when naloxone was administered by twice-daily injection [12]. Evidently, chronic or continuous

TABLE 1

CHANGES IN O_2 CONSUMPTION (ml/5 min) PRODUCED BY SUBCUTANEOUS INJECTION OF THREE DIFFERENT DOSES OF NALOXONE AND BY SALINE ALONE

Dosage (mg/kg)	n	Basline (mean ± SEM)	% Change from Baseline (mean ± SEM)	Comparison with Saline Control Group Dunnett's tD*
Saline	15	0.46 ± 0.03	+ 0.8% ± 1.8%	
0.7 NX	5	0.50 ± 0.06	−23.5% ± 3.6%	3.55, $p<0.01$
2.5 NX	5	0.37 ± 0.04	−28.9% ± 8.2%	4.40, $p<0.01$
11.4 NX	5	0.40 ± 0.02	+ 6.0% ± 9.2%	1.07, NS

*Test statistic for multiple comparisons with a single control group [8].

receptor blockade is necessary for these effects, since they did not occur after a single naloxone injection, even at very high doses, and even in combination with the mechanical trauma of osmotic minipumps. The only significant effects were decreases in O_2 consumption (opposite to abstinence syndrome). Such decreases have been previously reported and attributed to GABA-ergic side effects of naloxone [4].

The present data are consistent with the hypothesis that ongoing, daily endorphin-receptor stimulation conditions the nervous system to avert an abstinence-like state. Such a state might be constituted, for example, by hyperactivity of cyclic nucleotide-dependent biogenic amine transmission, which is normally suppressed by endorphin receptor stimulation [2, 3, 16, 20]. These same systems have been shown to become hyperactive during opiate abstinence [1, 9, 15, 16]. Recent experiments in our laboratory [11] show that, as with actual opiate abstinence syndrome, endorphin blockade syndrome is exacerbated by IBMX, which up-regulates cyclic nucleotides, and potently reversed by clonidine, which down-regulates noradrenergic activity.

One surprising finding in the present study was the sharp decline of behavioral and respiratory symptoms after two days of infusion. This is not attributable to cessation of naloxone infusion, since implanted minipumps have been shown to infuse drug solution at a constant rate for at least seven days [18,19]. One possible interpretation is that the nervous system makes a gradual dynamic adjustment to prolonged understimulation of endorphin receptors, just as it makes a gradual dynamic adjustment to prolonged overstimulation of these receptors during the development of opiate dependence. The same negative-feedback loop for regulating endorphinergic stimulation might conceivably account for both phenomena. It has been shown that prolonged exposure to opiate antagonists can cause an increase in opiate receptor number [25]. This is one possible compensatory mechanism which might account for recovery from symptoms produced by continuous receptor blockade.

The fact that prolonged naloxone exposure can produce such symptoms might have interesting implications regarding mechanisms of opiate dependence formation. There is some preliminary evidence for the existence of an endogenous opioid antagonist in morphine tolerant and dependent animals [7, 10, 13, 21, 22, 23] and in acupuncture-tolerant animals [7]. The effects of prolonged naloxone exposure raise the possibility that such an endogenous antagonist might contribute to the development of opiate dependence.

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