Pharmacology Biochemistry & Behavior, Vol. 25, pp. 989–993, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Clonidine Reverses the Behavioral and Respiratory Effects of Continuous Naloxone Infusion[1]

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Received 31 March 1986

MALIN, D. H., A. G. HEMPEL, R. J. EXLEY AND S. ADDINGTON. *Clonidine reverses the behavioral and respiratory effects of continuous naloxone infusion.* PHARMACOL BIOCHEM BEHAV 25(5) 989–993, 1986.—Opiate naive rats received 24 hours of continuous subcutaneous infusion of 0.67 mg/kg/hr naloxone via osmotic minipump. As in previous studies, this induced an opiate-abstinence-like syndrome of significantly increased oxygen consumption and behavioral signs (wet-dog shakes, abdominal writhes, etc.). Clonidine, which selectively reduces central noradrenergic activity, has been shown to reverse opiate abstinence syndrome. Subcutaneous injection of 0.033 and 0.01 mg/kg clonidine totally reversed the abstinence-like behaviors and respiratory activity induced by naloxone infusion. This constitutes an additional point of similarity between opiate abstinence syndrome and the "endorphin blockade syndrome" or withdrawal from endogenous opioids resulting from chronic naloxone treatment. It is consistent with the hypothesis that hyperactivity of central noradrenergic mechanisms may contribute to both phenomena.

Clonidine Naloxone Opiate antagonists Endorphins Opiate abstinence syndrome Respiration Adrenergic receptors

UNLIKE acute naloxone administration, chronic naloxone injections [14] or continuous naloxone infusion [15] induce an opiate-abstinence-like syndrome in the rat. Such typical abstinence signs as wet-dog shakes and abdominal writhes are noted, along with respiratory hyperactivity and failure to gain weight. As with actual opiate abstinence syndrome, these symptoms are reversible by low doses of morphine [14].

These results raise the question, to what extent does the "endorphin blockade syndrome" induced by chronic endorphin receptor blockade share the same underlying biochemical mechanisms as opiate abstinence syndrome? One important mechanism underlying abstinence syndrome appears to be hyperactivity of brain noradrenergic activity [21,27]. Opiate dependent animals exhibit increased firing rates of noradrenergic locus coeruleus neurons [1]. Likewise, there is increased norepinephrine turnover in brain tissue of opiate dependent rats and monkeys [5, 16, 22]. Consistent with this are the elevated levels of the norepinephrine metabolite MHPG detected in brain and plasma of opiate abstinent monkey [22] and in plasma of opiate abstinent human addict [4].

The alpha-2 adrenergic agonist clonidine potently and selectively reduces central noradrenergic activity [3,23]. DiStefano and Brown [6] report that clonidine reverses the increased tyrosine hydroxylase activity and adrenal epinephrine secretion that accompany opiate abstinence. Clonidine potently reverses opiate abstinence signs in dependent rats [1, 2, 8, 11, 20, 24, 25]. Clonidine has also proved useful for reversing withdrawal symptoms in human heroin addicts [13, 14, 26]. It was therefore of interest to determine whether clonidine would likewise reverse the abstinence-like signs induced by continuous endorphin blockade. Doses of 0.033 and 0.01 mg/kg were chosen for study since they corresponded respectively to the middle and lower ranges of the doses administered to opiate abstinent rats by Fielding *et al.* [11] and Meyer and Sparber [20].

EXPERIMENT 1

Method

Subjects were twenty male Sprague-Dawley rats weighing an average of 135 g with a range of 120–150 g. Throughout the experiment, all rats were maintained on ad lib food and water and on a twelve hour light and dark cycle. All rats were habituated to an oxygen consumption chamber once a day for four consecutive days. Ten rats were subcutaneously implanted with two Alzet 2001 osmotic minipumps filled with 50 mg/ml naloxone in saline. These rats were continuously infused with 0.67 mg/kg/hour naloxone. Ten other rats were

[1]Supported by a University of Houston—Clear Lake Organized Research Grant. Clonidine was donated by Boehringer-Ingelheim Inc. and naloxone was donated by Du Pont Laboratories, Wilmington, DE. The authors thank Leah Harter for editorial advice and assistance.

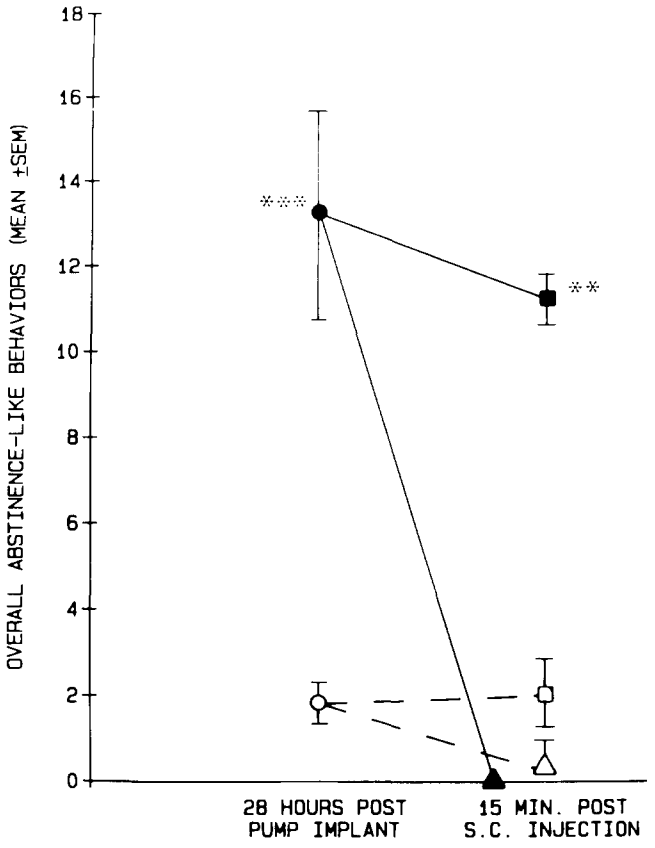

FIG. 1. Overall abstinence-like signs in 10 rats infused for 28 hours with 0.67 mg/kg/hr naloxone and in 10 rats infused with saline alone. Half of each group was retested after injection of saline SC, and the other half was retested after injection of 0.033 mg/kg clonidine SC (***$p<0.001$ compared with saline group; **$p<0.01$ compared with all other retested groups). Naloxone infused: (●) pre-injection; (▲) post 0.033 mg/kg clonidine SC; (■) post saline SC. Saline infused: (○) pre-injection; (△) post 0.033 mg/kg clonidine SC; (□) post saline SC.

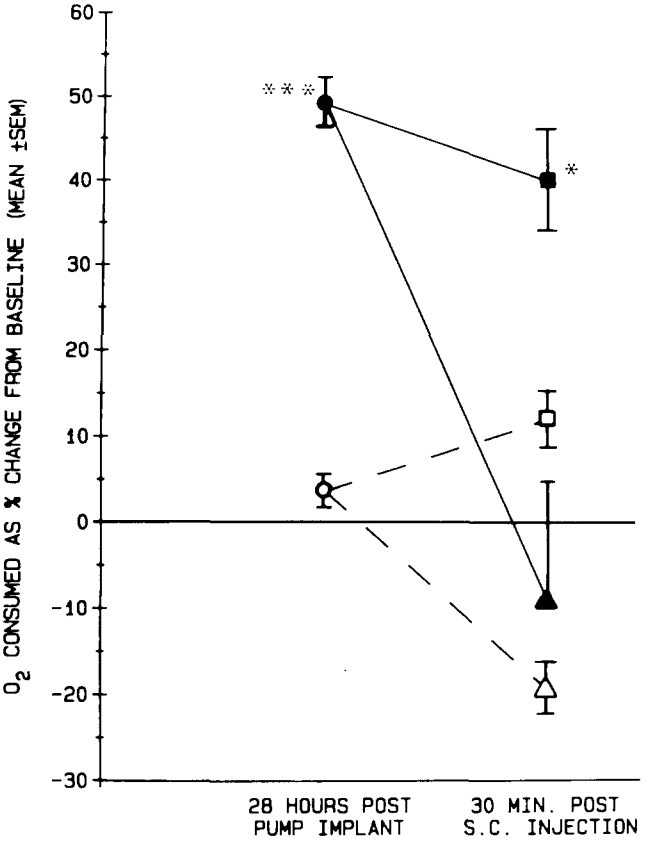

FIG. 2. Oxygen consumption as percentage change from pre-experiment baseline in 10 rats infused for 28 hours with 0.67 mg/kg/hr naloxone and in 10 rats infused with saline alone. Half of each group was then retested after injection of saline SC, and half was retested after injection of 0.033 mg/kg clonidine SC (***$p<0.001$ compared with saline-infused group; $p<0.05$ compared with all other retested groups). Naloxone and saline infused same as in Fig. 1.

implanted with osmotic minipumps filled with saline alone.

Prior to pump implantation, each rat was tested for baseline oxygen consumption rate by the method described by Malin *et al.* [18]. Unrestrained rats were placed in an oxygen-charged soda-lime chamber. A water-filled manometer indicated the amount of oxygen consumed during a 2.5 minute test.

At 28 hours after pump implantation (corresponding to 24 hours of full-rate infusion), all rats were observed for morphine-abstinence-like behaviors in a clear plastic open field with a grid floor. During a 15 minute test, these symptoms were scored on a standard checklist according to the criteria of Gianutsos, Drawbaugh, Hynes and Lal [12]. Symptoms included wet-dog shakes, head shakes, abdominal writhes and hind foot scratches. For purpose of statistical analysis, ptosis, teeth grinding, dyspnea, aggression and seminal ejaculation were grouped under the category of "other symptoms." The frequencies of all of the above symptoms were summed for each rat to determine the "overall symptoms" score for that animal. Following the observations, each rat was retested for oxygen consumption rate.

Half of the saline-infused and half of the naloxone-infused rats were then injected subcutaneously with 0.033 mg/kg clonidine. The remaining rats in each group were injected with an equivalent volume of saline alone. Fifteen minutes after injections, the behavioral observations and oxygen consumption measurements were repeated for all rats.

Results

After 28 hours of infusion, the naloxone-infused rats had significantly higher overall abstinence-like behavioral signs than the saline-infused controls, $t(18)=4.54$, $p<0.001$. As shown in Fig. 1, the naloxone group averaged 13.2±2.5 signs (mean±SEM), while the saline group averaged 1.8±0.3 signs. Wet-dog shakes, abdominal writhes, head shakes and scratches were all significantly elevated in the naloxone group. Following subcutaneous injections, the naloxone-infused rats injected with saline averaged 11.2±0.6 signs, while the naloxone-infused group injected with clonidine displayed no signs. The saline-infused rats injected with saline averaged 2.0±0.7 signs, while the saline-infused rats injected with clonidine averaged 0.2±0.1 signs. Two-way ANOVA of the post-injection data revealed a significant infusion effect (naloxone vs. saline), $F(1,16)=54.7$, $p<0.01$, a

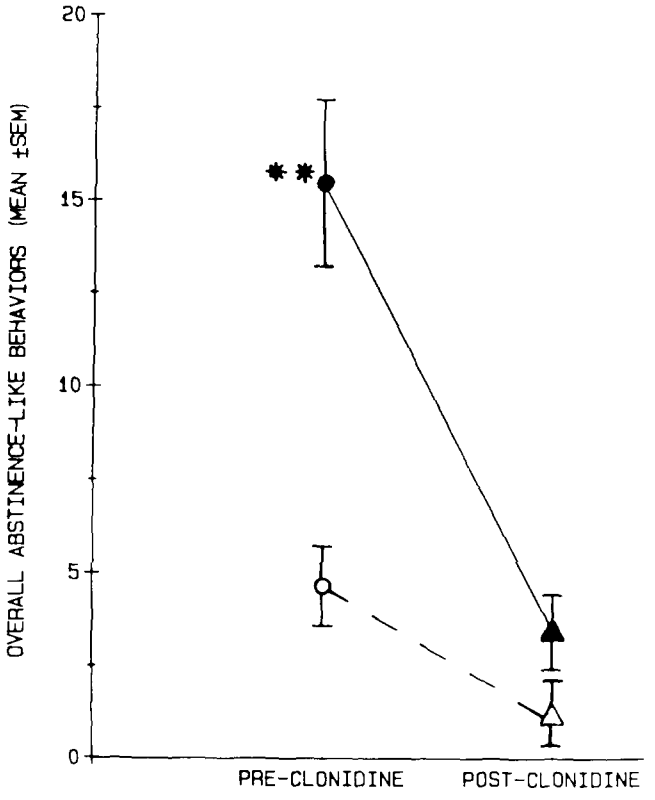

FIG. 3. Overall abstinence-like signs, before and after 0.01 mg/kg clonidine SC, in 5 rats infused for 28 hours with 0.67 mg/kg/hr naloxone and in 5 rats infused with saline alone (**$p<0.01$ compared with all other groups). Naloxone infused: (●) pre-injection; (▲) post 0.01 mg/kg clonidine SC. Saline infused: (○) pre-injection; (△) post 0.01 mg/kg clonidine SC.

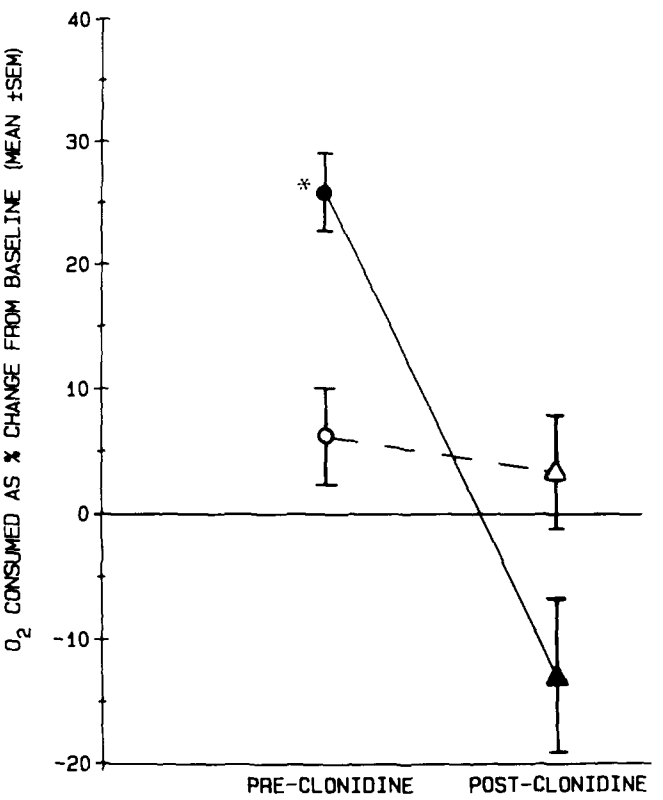

FIG. 4. Oxygen consumption as percentage change from pre-experimental baseline, before and after 0.01 mg/kg clonidine SC, in 5 rats infused for 52 hours with 0.67 mg/kg/hr naloxone and in 5 rats infused with saline alone (*$p<0.05$ compared with all other groups). Naloxone and saline infused same as in Fig. 3.

significant injection effect (clonidine vs. saline), $F(1,16)=114.2$, $p<0.01$, and a significant interaction effect (infusion × injection), $F(1,16)=59.7$, $p<0.01$.

Dunnett's procedure [12] for comparing a single group *post hoc* with several other groups showed that the group infused with naloxone and injected with saline only had significantly more abstinence-like signs than any of the other three groups, $p<0.01$.

After 28 hours of infusion, the naloxone-infused group showed a significantly greater increase above baseline oxygen consumption than the saline-infused controls, $t(18)=4.44$, $p<0.001$. As shown in Fig. 2, the naloxone group increased 49.0±2.3% above baseline, while the saline group increased only 3.6±1.1%. Following subcutaneous injections, the naloxone-infused rats injected with saline still had oxygen consumption elevated 40.0±6.4% above baseline, while the naloxone-infused rats injected with clonidine fell to 9.2±14.2% below baseline. Saline-infused rats injected with saline averaged 12.0±3.2% above baseline, while saline rats injected with clonidine averaged 19.5±3.6% below baseline. Two-way ANOVA of the post-injection data revealed significant effects of infusion (naloxone vs. saline), $F(1,16)=6.84$, $p<0.05$, and of injection (clonidine vs. saline), $F(1,16)=30.9$, $p<0.01$. The interaction effect was not significant, $F(1,16)=1.7$, NS.

Dunnett's procedure showed that the rats infused with naloxone and injected with saline only had significantly higher oxygen consumption than any of the other three groups, $p<0.05$.

EXPERIMENT 2

Method

This experiment tested the effects of a lower dose of clonidine. The same methods were employed as in Experiment 1, with the following exceptions. The subjects were ten male Sprague-Dawley rats with a mean weight of 160 g. Five rats were infused with 0.67 mg/kg/hr naloxone in normal saline, while five were infused with normal saline alone. At 28 hours after pump implantation, each rat was observed for abstinence-like behavioral signs for 15 minutes, was then injected subcutaneously with 0.01 mg/kg clonidine, and was retested at 15 minutes post-injection. (No rats were retested after saline injection, since that would be redundant with saline-retest groups in Experiment 1.) After 28 and 52 hours of infusion, each rat was tested for oxygen consumption for 2.5 minutes. After the 52 hour test, when the naloxone-infused rats reached a significant elevation over pre-experiment baseline, each rat was injected with 0.01 mg/kg clonidine, and was retested for oxygen consumption 30 minutes post-injection.

Results

As shown in Fig. 3, the naloxone-infused rats averaged 15.4±3.4 abstinence-like signs before clonidine and only 3.4±1.1 signs after clonidine. The saline-infused rats averaged 4.6±1.1 signs before clonidine and 1.0±0.8 signs after clonidine. Analysis of variance with one repeated measures variable indicated a significant infusion effect (naloxone vs. saline), $F(1,8)=14.9$, $p<0.01$, as well as a significant injection effect (clonidine vs. saline), $F(1,8)=43.0$, $p<0.01$. There was also a significant interaction effect (infusion × injection), $F(1,8)=12.5$, $p<0.01$.

Dunnett's procedure indicated that there were significantly more abstinence-like signs in the naloxone-infused rats prior to clonidine than in any of the other three conditions, $p<0.01$.

As shown in Fig. 4, the naloxone-infused rats increased their oxygen consumption to 25.5±3.5% above baseline, but fell to 13.2±6.4% below baseline after injection of 0.01 mg/kg clonidine. The saline-infused rats averaged only 6.0±3.6% above baseline before clonidine and 3.2±4.6% above baseline after clonidine. Analysis of variance indicated a significant infusion effect (naloxone vs. saline), $F(1,8)=37.7$, $p<0.01$, as well as a significant injection effect (clonidine vs. saline), $F(1,8)=38.7$, $p<0.01$. There was also a significant interaction between infusion and injection, $F(1,8)=32.4$, $p<0.01$.

Dunnett's procedure indicated that the oxygen consumption was significantly higher in the naloxone-infused rats prior to clonidine than in any of the other three conditions, $p<0.05$.

DISCUSSION

It appears that certain aspects of the opiate abstinence state can be elicited in opiate-free rats by endorphin receptor blockade. For example, Eisenberg has shown that naloxone [7] or naltrexone [9] treatment induces the sort of plasma corticosterone response seen during opiate abstinence. In the present study, as in previous studies from our laboratory [17,18], wet-dog shakes, abdominal writhes, head shakes and scratches were all significantly elevated in naloxone-infused rats. The present results indicate that naloxone-induced endorphin blockade syndrome, like the actual opiate abstinence syndrome, is reversible by small doses of clonidine. This is a further point of similarity between the two syndromes. Clonidine is believed to reverse opiate abstinence signs by reversing a state of noradrenergic hyperactivity [1,6]. The present results suggest that chronic endorphin blockade in otherwise normal organisms may induce a similar state of noradrenergic hyperactivity.

Chronic or continuous, but not acute, endorphin receptor blockade gradually induces behavioral irritability and increased metabolism [17,18]. Such changes appear to be totally reversible by reduction of central noradrenergic activity. These findings suggest the possibility that ongoing endorphin secretion maintains behavioral and physiological stability through long-term control over neurotransmitter systems, especially the noradrenergic system.

This hypothesis suggests that rebound hyperactivity of the same central noradrenergic mechanisms suppressed by clonidine might be expected to result in an opiate-abstinence-like phenomenon. In fact, an opiate-abstinence-like behavioral and respiratory syndrome does result following either abrupt [10] or yohimbine-precipitated [19] withdrawal from continuous subcutaneous clonidine infusion. The abstinence-like signs are reversible by both clonidine itself and morphine [10].

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