Pharmacology Biochemistry & Behavior, Vol. 9, pp. 319–325. Printed in the U.S.A.

Clonidine Antagonizes Naloxone-Induced Suppression of Conditioned Behavior and Body Weight Loss in Morphine-Dependent Rats[1]

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(Received 17 July 1977)

SPARBER, SHELDON B. AND DALE R. MEYER. *Clonidine antagonizes naloxone-induced suppression of conditioned behavior and body weight loss in morphine-dependent rats.* PHARMAC. BIOCHEM. BEHAV. 9(3) 319–325, 1978.—Clonidine's action on naloxone (Nx)-induced suppression of fixed-ratio (FR15) responding and body weight loss was studied in morphine (M)-dependent rats. Clonidine (10–70 μg/kg IP) injected 30 min prior to the behavioral session resulted in a dose-related suppression of operant behavior in M-naive animals. A small, but significant decrease (3–5%) in body weight was also observed at the higher doses of clonidine. More than twice as much weight loss, associated with diarrhea, was obtained when Nx (5.0 mg/kg IP) was administered to M-dependent animals. When clonidine (10–50 μg/kg IP) was administered prior to Nx, in M-dependent animals, the withdrawal-induced disruption of operant responding was significantly attenuated. Concurrent weight loss, which was significantly antagonized by 1 μg clonidine/kg, was decreased by as much as 40 percent. The degree of amelioration of withdrawal that was observed appeared to be inversely related to the dose of clonidine. The optimal dose was 10 μg/kg, which by itself was only partially behaviorally active. At higher doses, clonidine's blocking properties were less apparent as a result of its own potent behavioral suppressant and diuretic effects which masked its capacity to attenuate withdrawal. The data are discussed in relation to the application of operant technics for assessing drug treatment(s) designed to alter the severity of narcotic withdrawal.

Clonidine Morphine withdrawal Conditioned behavior Body weight

MANY of the effects of opiates or withdrawal from them appear to involve monoamine transmitter substances [19, 35, 42] and/or acetylcholine [11,14]. The antihypertensive agent clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline HCl) is thought to stimulate central α-adrenergic receptors [2,20], perhaps a subclass dubbed α_2 receptors [25] located on catecholaminergic cell bodies or processes, whose stimulation results in diminished cell firing [6] and diminished release of its transmitter [12,13]. It is thought that clonidine is capable of modulating cholinergic [10, 27, 33] and serotonergic activity as well, but these may be secondary to its direct action on NE cells [37].

Clonidine has been shown to possess certain pharmacologic properties similar to those of the narcotics. Paalzow [32] has described its antinociceptive activity in mice and rats, and its action on sympathetic preganglionic units in the cat has been observed to mimic the action of acute morphine (M) administration (D. Franz, personal communication). Since clonidine and M are both capable of inhibiting spontaneous activity of noradrenergic neurons [6,23], it seemed reasonable to examine the effects of clonidine for its ability to attenuate withdrawal symptoms in M-dependent rats.

Several laboratories have reported the disruptive effects of withdrawal from chronic M treatment upon operant behavior maintained by various schedules of reinforcement in monkeys [18, 22, 38] and rats [3, 15]. We have also reported that alterations in fixed ratio (FR) behavior in M-dependent rats correlated well with body weight loss when naloxone (Nx) was used to induce withdrawal [15]. Additionally, plasma corticosterone is greatly elevated in rats given low doses of Nx capable of suppressing FR behavior of rats made acutely tolerant/dependent by a single M injection or implanted with M pellets [31,36]. Acute, high doses of M (e.g. 30 mg/kg, IP) which have little behavioral suppressant action in M-pelleted, tolerant rats can prevent withdrawal-induced suppression of FR behavior of M-pelleted rats treated with an otherwise disruptive dose of Nx [7].

In the present report, we describe the results of experiments which indicate that acute clonidine administration

[1]Supported in part by USPHS grants DA00532 and MH08565. Preliminary reports of the capacity of clonidine to antagonize withdrawal in morphine-dependent rats were presented at the 1976 Fall Meeting, American Society for Pharmacology and Experimental Therapeutics (*Pharmacologist* 18(2): 236, 1976).

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prior to Nx-precipitated abstinence can also significantly attenuate the withdrawal-induced disruption of operant behavior in M-dependent rats. As an additional index of severity of withdrawal, body weight loss was also examined.

METHODS

Animals

Adult male Long-Evans hooded rats (280–310 g) were obtained from Simonsen Laboratories (Gilroy, CA). They were individually housed in air conditioned quarters (25°C and 50% humidity) under a 12 hr light-dark cycle (lights on from 0600 to 1800) for at least 2 weeks before use. Body weight was gradually reduced to 80% of free-feeding weight by a modified feeding schedule, and maintained thereafter. Water, but not food was available in their home cages ad lib. All animals were M-naive.

Apparatus

Standard operant conditioning boxes (Model No. 143-22; BRS/LVE, Beltsville, MD) were used. Each box was equipped with a lever and a dispenser for the delivery of 45 mg Noyes (Lancaster, NH) food pellets. External sound and light attenuation were provided by enclosing the box in an insulated and ventilated outer chamber which was equipped with a masking noise generator.

A house-light (15 W bulb) inside the box provided a low-level illumination during training and experimental sessions. All experimental events and contingencies were automatically controlled and recorded by a Nova-2 mini-computer (Data General) coupled with Interact System interfacing (BRS/LVE). Continuous records of behavior were also monitored on cumulative recorders (Ralph Gerbrands, Arlington, MA).

Training Procedure

Rats were trained to lever press for food pellets on a continuous schedule of reinforcement (CRF) for 3 days. During the next session each animal was placed under a fixed-ratio (FR2) schedule and left in the experimental chamber for 1 hr. During subsequent sessions the ratio requirement was slowly incremented until responding under a FR15 schedule was stable.

After initial training animals were acclimated to the experimental regimen. Throughout all experiments each animal was put into the operant chamber and allowed to lever press for 5 daily, 5 min periods (spaced 1 hr apart). During each 5 min epoch the responses emitted and reinforcers earned were recorded. After each period in the operant chamber, the animal was removed from the apparatus, weighed and placed back into its home cage during which time ad lib access to water, but not food, was available. Animals were habituated to saline injections for at least 2 days before use in experimental conditions.

Five min epochs were used in the protocol since we had observed previously that this interval was sufficient for assessing drugs' actions, over an extended period of time, without being confounded by satiation effects [15]. Using this procedure we could monitor the effects of clonidine and/or Nx for up to 4 hr after administration. The first 5 min epoch of each daily session served as a pre-injection control. Response rate during this period was used to confirm that all animals were responding at normal rates before control or experimental conditions were commenced. If an animal was not responding at its normal rate, a second control period was given before proceeding with the daily operant experimental session.

Effect of clonidine on FR responding in M-naive animals. To assess the effect of clonidine upon operant behavior, M-naive animals (N=5) were injected with either clonidine or saline prior to the daily behavioral session as described previously. Each animal served as its own control and drug effects were expressed in terms of control response rates. During the first 2 days baseline data were collected. Saline was administered at 30 and 10 min prior to the second 5 min epoch each day. During the experimental phases each animal received all 4 doses of clonidine. The various doses of clonidine were administered in a randomized block design, 30 min prior to the second epoch.

Effects of clonidine-Nx interaction in M-dependent animals. In order to assess the effect of clonidine upon Nx-precipitated abstinence, the 5 animals that were used previously were rendered M-dependent. During the first 2 days baseline data were collected as previously described. On the third day the animals were implanted with a single M pellet (75 mg base) which was formulated according to the method of Gibson and Tingstad [16]. Each animal was anesthetized with pentobarbital sodium (30 mg/kg, IP) and an M pellet was inserted under the skin in the shoulder region. The incision was closed with wound clips. The type of pellet which was used has been described previously (Type C) [29]. Twenty-four hr after implantation 2 days of baseline data were collected for use as control values. On Days 3–5 after implantation, clonidine (50 μg/kg), Nx (5.0 mg/kg), or both were administered to animals prior to the daily behavioral session. Each animal served as its own control and all drug effects were assessed in terms of control response rates. Three different drug conditions were assessed: (1) clonidine-saline (CS); (2) saline-Nx (SNx); and (3) clonidine-Nx (CNx). Clonidine (or saline) was administered 30 min before and Nx (or saline) was administered 10 min before the second 5 min epoch each day. All drug conditions were given to each animal during the 3 day period using a randomized block design.

The dose of clonidine (50 μg/kg) was chosen, on the basis of preliminary experiments, to represent a dose that would depress operant behavior to about 50% of control values for the entire 3 1/2 hr period (Epochs 2–5). The dose of Nx (5.0 mg/kg) was chosen since preliminary data indicated that this dose was highly effective in causing behavioral disruption in M-dependent animals (with the pellets utilized in these experiments) for at least 2 hr.

In order to assess the effect of lower doses of clonidine on Nx-precipitated abstinence, the experimental paradigm was replicated twice using 3 additional doses. For both replications each animal again served as its own control and all drug effects were assessed in terms of control reponse rates. The methodology that was used for the collection of control and experimental data was identical to that described for the initial experiment. After termination of the initial experiment, M pellets were removed, and animals were allowed to recover for 3 weeks before reuse. A similar recovery period was allowed between replications. The effect of Nx after reimplantation was comparable to that observed during the original experiment. This indicated that repeated exposure to M pellets did not systematically alter the degree of physical dependence which developed after each pellet implantation. For the first replication, animals were reimplanted with a single M pellet as described. Starting 24 hr after implantation 2 days of baseline data were collected to be used as control

values. On Days 3–5 after implantation, clonidine (30 μg/kg), Nx (5.0 mg/kg), or both were administered to each animal using a randomized block design. For the second replication, animals were reimplanted with a single M pellet. Starting 24 hr after implantation 2 days of baseline data were again collected for use as control values. On Days 3–5 after implantation, clonidine (10 μg/kg), Nx (5.0 mg/kg), or both were administered to each animal using a randomized block design. On Days 6–8, the same procedure was repeated, with the exception that clonidine (1.0 μg/kg) was given. Although 6–8 days elapsed between pellet implantation and the experiment using 1.0 μg clonidine/kg, the administration of saline followed by the same dose of Nx resulted in a disruption of operant behavior and body weight loss which was not significantly different from that observed 3–5 days after pellet implantation in the original experiment and the 2 replications. This verifies the relative constancy of physical dependence which is induced by this type of pellet [29] and the reliability of these measures of withdrawal [15] over the 8 day period.

The doses of clonidine that were used for the final replication were chosen on the basis of preliminary experiments, which indicated that clonidine in this dose range was behaviorally inactive as measured by the operant paradigm used.

Data Analysis

To obtain quantitative indices of the effects of clonidine and Nx (or both) upon operant behavior, the areas under the response-time curves for the last four 5 min epochs were measured. Integration of the area under the curve was calculated by the trapezoid method. Areas under the curve for Epochs 2–3 and 4–5 were calculated separately. The first two (5 min) epochs were taken to represent the acute abstinence reaction due to the direct effect of Nx upon opiate receptors. The second two epochs were considered to represent a recovery phase, where behavioral disruption was rapidly waning, and therefore difficult to clearly demonstrate further attenuation. Areas under the response-time curves were compared for the various drug conditions by analysis of variance.

Changes in body weight during daily operant sessions were calculated as the difference between initial weight (Epoch 1) and final weight (Epoch 5). Separate calculations were made for each animal throughout all experiments. Body weight changes for the control condition were calculated as the mean of the control sessions for each experiment. The difference in body weight during the control and experimental condition was compared using paired sample *t*-tests. In those experiments in which several drug conditions were assessed, multiple paired comparisons were made.

Drugs

M pellets were generously supplied by Dr. M. W. Adler, Temple University, Philadelphia, PA. Nx HCl was a gift from Endo Laboratories, Garden City, NY. Clonidine HCl was a gift from Boehringer Ingelheim, Elmsford, NY. Pentobarbital sodium was purchased from Sigma Chemical Co., St. Louis, MO. All drugs were dissolved in physiologic saline in concentrations such that all doses were administered IP in a volume of 1 ml/kg. Saline injections were given in an equal volume. Doses indicated pertain to the salts.

RESULTS

Effect of Clonidine on FR Responding in M-Naive Animals

The magnitude and duration of the behavioral disruption produced by clonidine was dose-related (Fig. 1). As illustrated, the peak effect of all doses was evident within 30 min after the injection. When 10 μg/kg was given, operant performance was initially disrupted to about 70% of control values, but recovery was complete by 1 1/2 hr. When 30 μg/kg was given, operant behavior was disrupted to a greater extent (to about 30% of control) and recovery was not complete until 3 1/2 hr after the injection. When higher doses (50 and 70 μg/kg) were administered the initial behavioral effect was comparable but greatly prolonged. The duration of action of these doses exceeded 3 1/2 hr and recovery was not complete at the time behavioral testing was terminated.

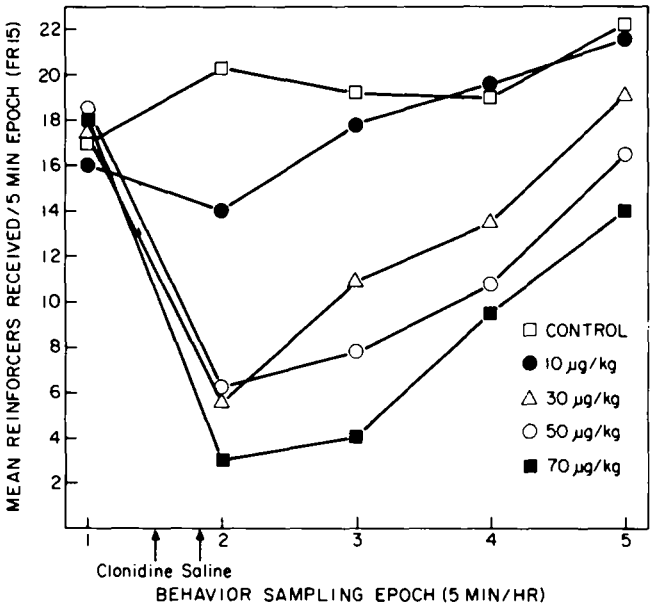

FIG. 1. Mean number of reinforcers earned throughout daily behavioral sessions. Each operant period represents a 5-min epoch spaced 1 hr apart. The data shown represent control and either 10, 30, 50 or 70 μg clonidine/kg. Each animal (N=5) served as its own control and all 4 doses of clonidine were administered in a randomized block design. During control sessions, saline was administered (IP) 30 and 10 min prior to the 2nd epoch. During drug sessions, clonidine was administered (IP) 30 min before Epoch 2, and saline was administered 10 min prior to Epoch 2. For clarity, estimates of variability were left out of the figure. At lower doses of clonidine (10 μg and 30 μg/kg) and saline, standard errors of the means were approximately 10% of the means. At the two higher doses of clonidine the SEM were approximately 5% of the means.

A concurrent loss in body weight was also observed after clonidine administration, which did not appear to be correlated with its behavioral activity (not shown). All animals receiving clonidine in doses which were behaviorally active (>10 μg/kg) lost approximately 10 g during behavioral testing. Peak weight loss was observed within 35 min (after the 2nd epoch), but most animals continued to lose weight for several hr. The absence of any apparent correlation between behavioral suppression and weight loss suggests that the drug effects may not be totally dependent upon each other.

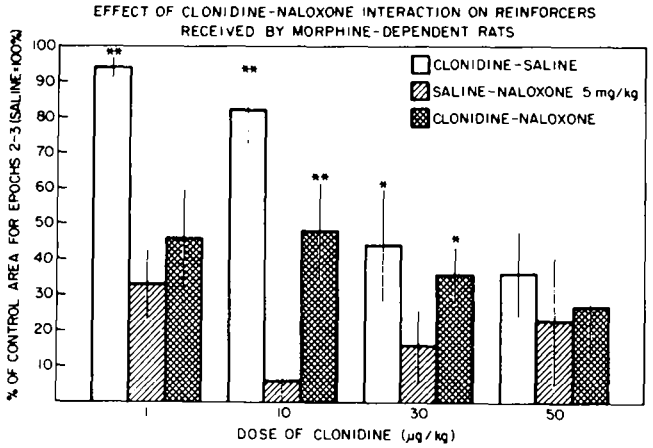

FIG. 2. The effect of clonidine pretreatment upon naloxone-precipitated suppression of operant behavior in morphine-dependent rats. The data are expressed as a percentage of control values (saline) for mean reinforcers earned during the first two, 5-min epochs (Epochs 2–3 of Fig. 1) following drug administration. Each bar represents the mean (±SE) from 5 animals. The data illustrated represent the clonidine-saline (CS), saline-naloxone (SNx) and clonidine-naloxone (CNx) treatments for 3 experiments in which 4 different doses of clonidine were given. For all experiments each animal served as its own control and all 3 drug treatments were administered in a randomized block design. Clonidine (or saline) was administered 30 min before Epoch 2, and saline (or naloxone) was administered 10 min before Epoch 2. Significant differences from the SNx condition are indicated, $*p<0.05$, $**p<0.01$ (one-way analysis of variance).

Clonidine-induced weight loss may have been the result of excessive urination, since previous investigators have demonstrated that the drug does possess mild diuretic properties [5,8]. Drug-induced diuresis is plausible, although the apparent lack of dose-dependency is inexplicable.

Effect of Clonidine-Nx Interaction in M-Dependent Rats

The data shown in Fig. 2 illustrate the effect of clonidine pretreatment upon operant responding during the first two 5-min epochs following the administration of Nx. The results of all 3 experiments are shown in the figure. When clonidine was administered to M-dependent animals, a dose-related suppression of operant behavior was observed starting 1/2 hr after drug administration. Low doses of clonidine (1 and 10 μg/kg) were behaviorally inactive, but higher doses (30 and 50 μg/kg) disrupted operant responding to about 45% and 35% of control values, respectively. Although not shown in Fig. 2, clonidine's behavioral action in M-dependent rats was of similar magnitude and duration for the remaining 2 behavior sampling epochs as that observed in the M-naive condition (see Fig. 1). The administration of Nx alone suppressed operant responding about 70–90% during the 1 1/4 hr following its administration to the M-dependent rats. However, the suppression caused by Nx was of a shorter duration than the highest dose of clonidine. Within 2–3 hr, behavior was recovering rapidly towards baseline rates.

When clonidine was administered prior to Nx, the apparent amelioration of withdrawal distress was inversely related to the pretreatment dose. With high doses of clonidine, the drug's blocking properties were less apparent as a result of its own pronounced behavioral effect. Analysis of the data for the first experiment, when 50 μg clonidine/kg was administered, indicated that the effects of clonidine and Nx were not additive, even though demonstrable antagonism of withdrawal was not evident. Operant responding during all 3 treatment combinations was significantly below control values ($p<0.01$). Responding under each drug condition was approximately 20–30% of control response rates. Analysis of the data from the second experiment, when 30 μg clinidine/kg was administered, indicated that at a lower dose clonidine's blocking effect could be observed. Although 30 μg clonidine/kg suppressed FR15 behavior to about 40% of control, pretreatment with this dose of clonidine prevented further behavioral suppression by a dose of Nx which itself suppressed responding to 15% of control rates. Mean number of reinforcers earned during the CS and CNx conditions did not differ, but were higher than those earned during the SNx condition ($p<0.05$).

When 10 μg clonidine/kg was administered, the drug's blocking properties were more apparent. This dose of clonidine was itself ineffective in significantly altering FR15 behavior in M-dependent rats. After Nx administration the FR15 behavior dropped to 5–10% of control during this replication. Pretreatment with 10 μg clonidine/kg 15 min prior to injecting Nx significantly attenuated ($p<0.005$) the disruption caused by the antagonist. Rats responded at 50% of their control rate during Epochs 2 and 3. Mean reinforcers earned during the CNx condition were about 8 times greater than that during the SNx condition. The lowest dose of clonidine (1 μg/kg) was not capable of significantly attenuating the behavioral disruptive effects of the high dose of Nx, which under both conditions (SNx, CNx) suppressed responding about 70% ($p<0.01$) when administered 6–8 days after pellet implantation.

The data shown in Fig. 3 illustrate the effect of clonidine pretreatment upon Nx-precipitated weight loss during behavioral testing. The results of all 3 experiments are shown in the figure. Following the administration of Nx there was an almost immediate precipitous loss in body weight. The peak effect was evident within 1 1/4 hr (2 epochs), although animals continued to lose weight throughout the behavioral session. Diarrhea was observed in most animals while transporting them from their home cages to the operant chambers.

Analysis of the data from the first experiment, when 50 μg clonidine/kg was administered, indicated that the effects of clonidine and Nx were not additive, even though demonstrable antagonism of Nx-precipitated weight loss was not observed. After Nx was given, both pretreated and unpretreated rats lost 14–17 g during the 3 1/2 hr of behavioral testing. Clonidine treatment itself resulted in a 9 g loss in body weight. The absence of additivity would suggest that although clonidine was not capable of significantly reducing weight loss, a minimal degree of blocking activity was present. This hypothesis is supported by the fact that lower doses of clonidine were more effective in blocking Nx-precipitated weight loss. Additionally, diarrhea was not observed in any animals pretreated with clonidine. Analysis of the data from the second experiment, when 30 μg clonidine/kg was administered, indicated that at a lower dose the drug's blocking effect could be readily observed. Weight loss in clonidine pretreated rats (CNx) was substantially less ($p<0.05$) than that observed in unpretreated controls after Nx was injected (SNx). Even though this dose of clonidine caused an 8–10 g loss, pretreatment with this dose reduced Nx-precipitated weight loss by about 25%. Analysis of data

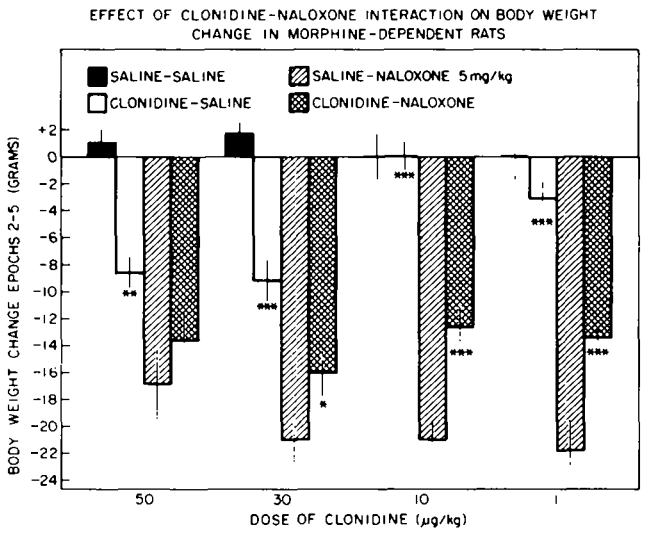

FIG. 3. The effect of clonidine pretreatment upon naloxone-precipitated weight loss (Epochs 2–5) in morphine-dependent rats. Each bar represents the mean (±SE) of 5 animals. The data illustrated represent the control (saline-saline), clonidine-saline (CS), saline-naloxone (SNx) and clonidine-naloxone (CNx) treatments for 3 experiments in which 4 different doses of clonidine were given. The experimental design and drug administration protocol are described in Fig. 2. Body weight loss during the 3 1/4 hr after Nx was given in the SNx condition averaged 8–10% of body weight. Significant differences from the SNx condition are indicated; $*p<0.05$, $**p<0.01$, $***p<0.005$ (two-tailed t-test for related samples).

from the third experiment indicated that when lower doses of clonidine (1 and 10 μg/kg) were given this effect was even more apparent. At these doses, clonidine itself had no effect upon body weight. When animals were given either 1 or 10 μg clonidine/kg, weight loss during the CNx and SNx treatments was significant, but demonstrably less ($p<0.005$) during the CNx condition. Pretreatment with 10 μg clonidine/kg reduced Nx-precipitated weight loss by 38%. One μg clonidine/kg before Nx reduced precipitated weight loss by approximately 40%.

DISCUSSION

Low to moderate doses of clonidine have been reported to produce sedation [9, 20, 21]. Cursory observations made upon picking the rats up 15 min after clonidine treatment, for their saline or naloxone injections and afterward for weighing, suggested this drug produced a calming effect. The rats were more easily handled by the experimenter. Acute administration of clonidine has been reported to disrupt avoidance behavior [26] and operant behavior maintained under a DRL schedule of reinforcement [39]. It has also been reported to decrease locomotor behavior [27,39] and prolong chloral hydrate- and/or hexobarbital-induced sleeping time [26]. When used clinically as an antihypertensive agent, sedation and depression has been observed [24,34].

The results of the first experiment, in which the dose-response characteristics of clonidine were determined, indicated that prolonged behavioral disruption could be observed with only 30 μg clonidine/kg in rats. Additionally, doses greater than 30 μg/kg can induce diuresis [8] probably as a result of a suppression of antidiuretic hormone release from the pituitary [5]. However, although maximal weight loss was observed within 35 min of administering clonidine to M-naive rats, its behavioral suppressant action which resulted in diminished food (reinforcers) intake cannot be discounted as a contributory factor [15].

The data from Fig. 3 show increasing body weight differences between SNx and CNx treatment as the dose of clonidine is decreased. It therefore appears that clonidine's blocking action on body weight loss is inversely proportional to the dose, as is the case with clonidine's attenuation of behavioral suppression. However, if at each dose the weight loss attributable to clonidine alone is subtracted from the weight loss occurring during CNx treatment, it can be seen that the remaining weight loss is markedly less than that which occurs during SNx treatment. This effect is greatest at the highest dose of clonidine, and diminishes as the dose is decreased. For example, in the experiment using 50 μg clonidine/kg, 5 mg Nx/kg caused a 17 g loss in body weight, but in the presence of clonidine, the additional weight loss in the CNx condition above that produced by clonidine alone amounted to only 5 g. This is a reduction in weight loss induced by Nx of 71%. Thirty μg clonidine/kg reduced Nx-induced weight loss by 66%, and 10 and 1 μg/kg reduced weight loss by 38% and 45%, respectively, when clonidine's effect in the CNx treatment is accounted for.

As an index of the severity of M-abstinence syndrome, suppression of operant behavior appears to be more sensitive than body weight loss. The more sensitive measure of dependence, manifest as a symptom of withdrawal, would therefore appear at lower doses of NX and continue to be observable for a longer period of time as dependence dissipates. Additionally, the more sensitive measure would be less amenable to antagonism or prevention by opiates or other pharmacologic agents which can ameliorate withdrawal and/or maintain dependence. For example, Gellert and Sparber [15] have previously demonstrated that semiweekly injections of Nx were capable of disrupting FR operant behavior in rats for up to 4 weeks after implantation of a single M pellet. Body weight loss in these same animals was significant only up to 3 weeks after implantation. Further evidence that operant behavior is a more sensitive index for characterizing M abstinence can be seen from our experiments with clonidine. A behaviorally inactive dose of clonidine as low as 1 μg/kg significantly attenuated Nx-precipitated weight loss. The next higher dose (10 μg/kg) resulted in a comparable attenuation of weight loss, as well as a significant attenuation of behavioral disruption caused by Nx. In other words, symptoms which are the first to appear seem to be the last to disappear as dependence develops and dissipates. These symptoms tend to be less susceptible to amelioration by pharmacologic means.

The maximal behavioral suppressant effect of Nx in this type of operant paradigm has been previously shown to occur between 20 and 25 min after IP administration [15]. Therefore, by the fourth and fifth epochs (2 1/4 to 3 1/4 hr after Nx) the response rates of SNx-treated animals were returning to pre-drug values. Because the differences between SNx and control responding during the last 2 epochs were much less than those during Epochs 2 and 3, an attenuation of Nx's diminishing suppressant effect by clonidine could not be detected. Perhaps clonidine's depressant properties are unmasked at this later time when the amount of Nx present in brain falls below a threshold level [4]. In addition, it is possible that clonidine exerts its block-

ing effect by interacting with the abstinence state at the cellular level during an initial primary stage. A secondary and subsequent stage of abstinence which may involve general stress or conditioned aversive stimuli, rather than direct biochemical or physiological consequences of withdrawal may be unaffected by clonidine.

However, if clonidine is acting directly upon the neuronal substrates responsible for withdrawal, it is probably on receptors other than the so-called opiate receptor. Using washed guinea pig brain membranes as a source of opiate receptors, and ^{3}H-etorphine as the primary ligand, clonidine (10^{-7} to 10^{-5}M) had no effect on etorphine binding (A. Goldstein, personal communication).

The effectiveness of clonidine pretreatment in ameliorating abstinence distress was similar to the drug effect we reported previously, when a symptom profile was used to characterize the severity of the abstinence syndrome [30]. In those experiments, we found that blockade of Nx-precipitated abstinence symptoms was positively related to the dose of clonidine used. When 50 μg clonidine/kg was given prior to Nx, 7 out of a possible 11 symptoms were significantly suppressed. The overall severity of the abstinence syndrome was reduced by approximately 60%. On the other hand, the data from the present experiments indicate that clonidine's effectiveness in blocking acute abstinence is inversely related to the dose used. The ephemeral nature of clonidine's activity is a function of its own potent effects. At high doses the behavioral activity of clonidine was sufficient to mask its withdrawal-blocking properties. The probability that clonidine's effect upon withdrawal is not related to a generalized sedative action is supported by the demonstration that blockade of Nx-precipitated abstinence is marked at doses of clonidine which are behaviorally inactive.

Most previous investigations of narcotic abstinence have looked at symptoms which essentially occur only during withdrawal (e.g., wet-dog shakes, teeth chattering, ptosis, jumping) to assess the degree of dependence. However, when pharmacologic agents are used with the intent to alleviate abstinence distress, the disappearance or amelioration of symptoms which have a zero-baseline of occurrence in the non-withdrawn animal does not necessarily indicate a specific or selective opiate-like action [22]. Instead, the animal may not display such symptoms after treatment with a drug because of a general stimulant or depressant effect, which has nothing to do with the underlying physiological mechanisms of dependence and/or the withdrawal syndrome. It would seem that an assessment of a potential therapeutic agent should involve examining its effects on symptoms or behavior normally present in the animal. For example, we have looked at clonidine's effect on operant behavior in both M-tolerant and drug-naive rats. By assessing clonidine's depressant effect on operant performance in non-withdrawn animals, it has been possible to achieve attenuation of Nx-precipitated behavioral disruption and weight loss at doses which have negligible disruptive effects. On the other hand, Tseng *et al.* [41] studied the influence of clonidine on the Nx-induced withdrawal signs of escape attempts and precipitated shakes on M-dependent rats. They showed that injection of 100–400 μg clonidine/kg IP, or 5 and 15 μg, intraventricularly, decreased the frequency of precipitated shakes and potentiated the number of escape attempts. These very high doses would certainly have rendered the animals incapable of engaging in an operant which was initially suppressed as a consequence of withdrawal.

Since wet-dog shakes may be diminished in severely withdrawn rats, it would appear that very high doses of clonidine can augment symptoms of withdrawal. This would be predicted if excessive concentrations of clonidine gained access to α_1 or post-synaptic α-receptors. If the withdrawal syndrome is associated in some way with excess firing and release by NE-containing neurons, low doses of clonidine would suppress this effect via a presynaptic action. However, no matter how inhibited the NE-containing cells are, clonidine, in high enough doses can mimic the effects of the transmitter (NE) whose release it is blocking. This prediction is somewhat analogous to the situation in which the dopamine agonist apomorphine is effective in alleviating clinical symptoms of both Parkinson's disease and Huntington's Chorea. The former is generally accepted as being caused by degeneration of nigrostriatal dopaminergic cells resulting in diminished dopaminergic stimulation within the striatum (and elsewhere) while the latter appears related, in part, to excessive dopaminergic stimulation. Without presynaptic terminals to inhibit (Parkinsonism), apomorphine can directly stimulate postsynaptic receptors. However, the constraints of the side effects of apomorphine in man also limit the doses given to patients with Huntington's Chorea. Since the presynaptic (autoreceptors) appear to be more easily accessible to exogenous agonists (drugs) or more sensitive to their dopaminergic action [1], it is not surprising that low doses of apomorphine can alleviate abnormal involuntary movements in Huntington patients [40].

In order to circumvent the problems that are associated with clonidine's potent behavioral effects it may be necessary to induce tolerance to this property before future investigations of the behavioral manifestations of M abstinence can be conducted. Preliminary experiments indicate that when clonidine is administered chronically, significant tolerance to its behavioral depressant properties develops within 1–2 weeks [28]. In clonidine-tolerant animals it may be possible to administer higher doses of the drug in order to demonstrate a more complete blockade of Nx-precipitated behavioral disruption. It remains to be ascertained whether or not tolerance to the drug's blocking properties develops as well.

During revision of this manuscript a report appeared in which clonidine has been utilized to treat withdrawing opiate addicts who had been maintained on methadone for extended periods [17]. All 5 subjects showed signs of opiate abstinence at least 36 hr into phased withdrawal. All patients reported dramatic relief of distress upon treatment with clonidine (5 μg/kg). The fact that spontaneous withdrawal symptoms could be alleviated in humans speaks to the generality of our observations and against the possibility that a direct antagonism of an Nx-specific effect, rather than attenuation of the opiate withdrawal syndrome, is responsible for clonidine's actions we report herein.

ACKNOWLEDGMENT

The technical assistance of B. Colleli in carrying out these experiments is gratefully acknowledged.

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