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Tolerance and Dependence after Chronic Administration of Clonidine to the Rat[1]

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MEYER, D. R., R. EL-AZHARY, D. WS. BIERER, S. K. HANSON, M. S. ROBBINS AND S. B. SPARBER. *Tolerance and dependence after chronic administration of clonidine to the rat.* PHARMAC. BIOCHEM. BEHAV. 7(3) 227–231, 1977. – Acute administration of clonidine (10–70 μg/kg, IP) disrupted operant behavior maintained by a fixed ratio schedule of reinforcement [1]. When chronically administered (100 μg/kg, IP and 3 μg/ml in drinking water) tolerance to the behavioral depressant effect developed within a few days and was complete by 14 days. Abrupt termination of drug treatment in tolerant rats resulted in an abstinence reaction which was characterized by suppression of operant performance for as long as one week. These results demonstrated the development of tolerance to and dependence on clonidine in rats. These behavioral observations in rats may be related to rebound hypertension and irritability of patients given this α-adrenergic agonist for treatment of hypertension.

Clonidine Tolerance Dependence Withdrawal Chronic Operant Behavior

SINCE the discovery of the hypotensive properties of clonidine (2-(2,6-dichlorophenylamine)-2-imidazoline), the drug has been widely used to treat patients who are refractory to other types of antihypertensive therapy [2]. Despite the fact that chronic maintenance with clonidine lowers blood pressure with little or no orthostatic or postural hypotension, side effects such as sedation have been reported [5,11]. Clinical reports suggest that side effects may be minimized by lowering the maintenance dose [2] but abrupt termination of treatment may result in a hypertensive crisis and irritability [4].

Despite the frequency of these clinical observations, systematic investigations have not been conducted to determine the behavioral manifestations of chronically administered clonidine in experimental animals. Earlier reports by Laverty and his associates [6,7] indicated that tolerance to clonidine's sedative properties may occur under some circumstances; and prolonged treatment often resulted in marked irritability and aggression. However, behavioral measures were not rigorously quantified, and most of their conclusions were based upon gross observation. Our interest in clonidine's effect upon operant behavior was due to reports that it possessed analgesic properties [10]. We observed that treatment of morphine-dependent rats with clonidine prior to naloxone, resulted in significant attenuation of withdrawal signs, including the disruption of operant behavior [1] and weight loss [8]. In the present communication we report the effect of chronically administered clonidine upon food-reinforced operant behavior in rats in which tolerance to and withdrawal from clonidine was observed.

METHOD

Animals

Adult male Long-Evans hooded rats were obtained from Simonsen Laboratories (Gilroy, CA) and individually housed in air-conditioned quarters (25°C and 50% humidity) under a 12 hr light-dark cycle (lights on from 0600 to 1800). All animals were originally trained to lever press for food under a fixed ratio (FR) schedule of reinforcement and used in a previous perfusion experiment, in which radiolabeled dopamine was infused into the caudate nucleus during behavioral testing. During this study animals had received a single injection of morphine and/or naloxone prior to infusion. Changes in catecholamine turnover were correlated with alterations in behavior. The rats had not been used for any other experimentation for at least 4–6 months. During the interim they were maintained at free-feeding weight. The present experiments were initiated after animals were food-deprived to 80% of their free-feeding weight (300–400 g). Water was available ad lib.

Apparatus

Standard operant conditioning chambers (Model No. 143–22; BRS/LVE, Beltsville, MD) were used. Each chamber was equipped with a lever and a dispenser for the

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delivery of 45 mg food pellets (Noyes Co., Lancaster, NH). External sound and light attenuation was provided by placing the chambers in insulated and ventilated environmental enclosures (BRS/LVE, Model No. 132–02) which were supplied with masking noise (20–20,000 Hz).

All experimental contingencies were automatically controlled and recorded by a Nova 2110 minicomputer (Data General Co., Southboro, MA) connected to an Interact System (BRS/LVE). Continuous records of behavior were also monitored on cumulative recorders (Ralph Gerbrands, Arlington, MA).

Clonidine Treatment

Rats were reshaped to lever press for food pellets on a continuous schedule of reinforcement. The FR requirement was incremented until rats responded at a stable rate on a FR 20 schedule. One hour behavioral sessions were conducted daily and data were collected between 1200 and 1400 hr. Each animal served as its own control and drug effects were expressed in terms of control response rates.

On Days 1–2 baseline data were collected in which saline (1 ml/kg, IP) was administered 15 min prior to testing. For the next 35 days the rats were maintained on a low concentration of clonidine in their drinking water (3 μg/ml) in order to facilitate the development of tolerance [7], and injected with clonidine (100 μg/kg, IP) daily, 15 min prior to behavioral testing. Normal fluid consumption was not affected and all rats ingested between 250–350 μg of clonidine/kg/day. The challenge dose of clonidine was chosen on the basis of previous experimentation [1] which indicated that acute administration of moderate to high doses (50–70 μg/kg) of the drug would disrupt operant behavior for several hours. During withdrawal, drug administration was discontinued and animals received only saline (1 ml/kg, IP) and drug-free water. All statistical analyses were performed by *t*-test for related samples. Drugs were administered as their hydrochloride salt.

RESULTS

Tolerance

Chronic administration of clonidine resulted in tolerance to the behavioral depressant effects within a few days. Tolerance was complete within 2 weeks and total responding during the behavioral session was not significantly different from control values. On the first day of clonidine treatment, animals showed an 80% suppression of operant behavior during the hour session (Fig. 1). By the fifth day significant tolerance was evident; responding had returned to approximately 50% of control rates. By Day 10 operant responding was still significantly depressed but response rates had returned to about 75% of control. The duration of action was approximately 30 min and animals were responding at control rates during the latter half of the operant session. By the fourteenth day of treatment, response rates had returned to 92% of control and clonidine was behaviorally disruptive only during the first 15 min of the session.

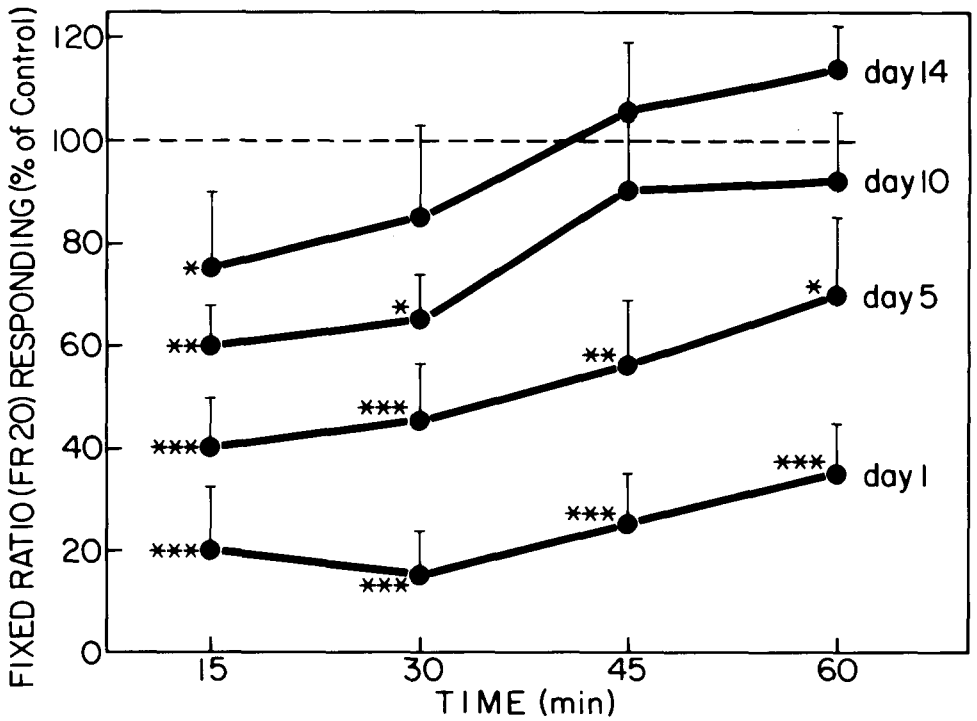

FIG. 1. Duration of drug-induced suppression of operant behavior maintained by a fixed ratio (FR 20) schedule of reinforcement in rats maintained on clonidine in drinking water (3 μg/ml) and injected with clonidine (100 μg/kg, IP) daily, 15 min prior to testing. Data shown represent responses emitted (% of control) during each 15 min segment of a 1 hr behavioral session for Days 1, 5, 10 and 14 of the tolerance phase. Each point illustrates mean ± SE for 3 animals. Mean control response rates (2 days; responses/min) for individual animals were 88.5, 63 and 57.5. Significant differences from control values are indicated: $*p<0.05$, $**p<0.025$, $***p<0.01$ (two-tailed *t*-test for related samples).

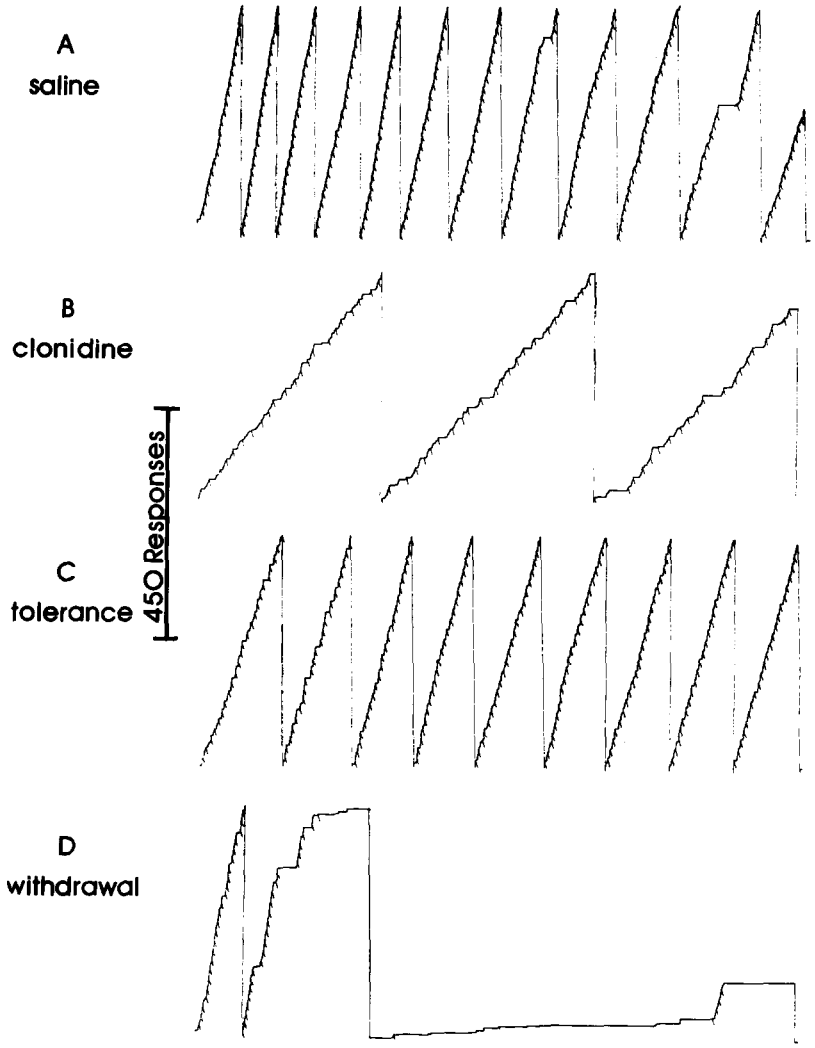

FIG. 2. Time course of withdrawal following the abrupt termination of drug treatment in clonidine-tolerant rats. Data shown represent response emitted (% of control) during each 15 min segment of a 1 hr behavioral session for Days 0, 1, 3, 5, 7 and 9 of withdrawal phase. Each point illustrates mean ± SE for 3 animals. (See legend to Fig. 1 for control response rates.) Significant differences from control values are indicated: $*p<0.05$, $**p<0.025$, $***p<0.01$ (two-tailed t-test for related samples).

Cumulative records of a representative rat are shown in Fig. 2. Baseline responses (FR 20 schedule) averaged 90 responses/min (Fig. 2a). Initial clonidine administration resulted in decreased response rates as well as increased prerun pauses and interresponse times. However, the latter two measures were not statistically analyzed and group data are not shown. After 2 weeks of treatment, response rates returned to baseline values (Fig. 2c).

Since we previously observed that clonidine can attenuate naloxone-precipitated withdrawal [1,8], we reasoned that tolerance to clonidine's action might be attended by an opiate-like dependence. We therefore administered naloxone to these animals to determine if a disruption of the operant, shown to be a sensitive indication of dependence upon morphine [3] would ensue. Each animal received 3 doses of naloxone and each dose was replicated twice. The narcotic antagonist was given daily, 10 min prior to behavioral testing. Naloxone (0.5, 1.0 and 2.0 mg/kg, IP) administered during the next 9 days of clonidine treatment (Days 15–23) failed to produce a systematic disruption of operant behavior in clonidine-tolerant rats. Although one animal was grossly affected by the 2 higher doses of naloxone, behavior of the other 2 animals was not suppressed and group response rates were not significantly different from control (data not shown).

Withdrawal

Thirty-five days after initiating treatment, clonidine administration was terminated. Abstinence was manifest as a disruption of operant behavior. A 50% suppression of operant behavior was observed on Day 1 into withdrawal.

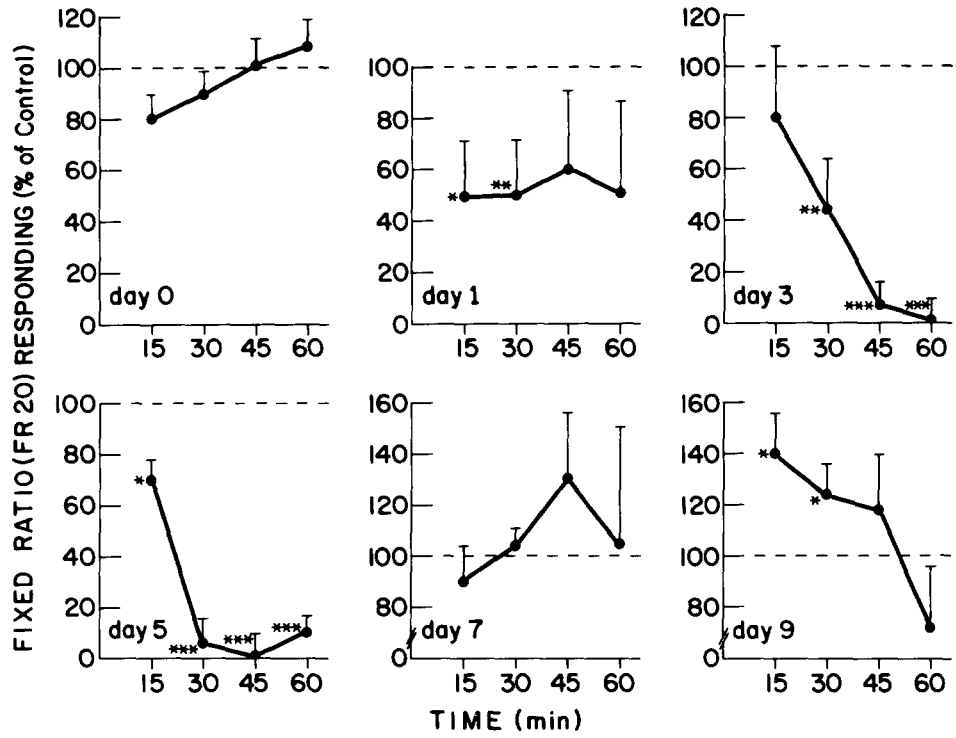

FIG. 3. Tolerance and withdrawal following chronic administration of clonidine (100 μg/kg, IP) as measured by operant behavior maintained on a fixed ratio (FR 20) schedule of reinforcement. Cumulative records of one animal during 1 hr behavioral sessions following saline injection, control baseline (A); Day 1 of clonidine treatment (B); Day 14 of clonidine treatment (C); and 5 days into clonidine withdrawal (D) are shown. Saline during control or withdrawal phases or clonidine was administered 15 min prior to testing.

Three days into withdrawal, normal response rates were maintained for the first 10 min, after which responding dramatically declined. The severity of abstinence peaked within 3 to 5 days after drug withdrawal and lasted for approximately 6 days. During peak withdrawal, operant behavior was reduced to 20–40% of control rates. The abstinence reaction subsided by Days 7–9 and total responding during the hour session was not significantly different from control values. However, the behavioral manifestations of withdrawal persisted, and the significant elevation in responding that was observed during the first half of the behavioral session on Days 8–9 may correspond to a mild protracted abstinence phenomenon (Fig. 3). No significant body weight loss was observed during the withdrawal phase. All animals maintained their weight at 80% of free-feeding levels throughout the experiment. Figure 2d shows an example of a cumulative record of an animal 5 days into withdrawal. Responding was at 80% during the first 15 min into the session and was followed by abrupt termination of the operant.

DISCUSSION

Clonidine (100 μg/kg) caused a significant suppression of operant behavior within 15 min after administration. Similar acute effects of clonidine have been reported by Colelli *et al.* [1] and Tilson *et al.* [12]. Continued administration resulted in a gradual dimunition of the disruptive effects of clonidine upon operant behavior and after 14 days animals were responding at control rates. Although tolerance to the sedative and hypotensive properties of the drug have been reported in humans [9] and rats [7], this is the first conclusive demonstration of tolerance following chronic clonidine administration in experimental animals in which behavioral measures were objectively quantified.

Since similar doses have been reported to cause hypotension in rats [7], it may be argued that the behavioral action we observed was through such a mechanism. However, this seems unlikely since we have observed significant behavioral suppression at doses which did not cause hypotension [1].

Abrupt termination of drug treatment caused marked withdrawal symptoms as evidenced by the suppression of operant behavior. The abstinence reaction reached its peak during the third to fifth day and lasted for approximately 6 days. By the seventh day responding returned to baseline values and signs of acute withdrawal were no longer apparent. This behavioral disruption differed qualitatively from that observed during chronic treatment. During the development of tolerance animals responded at decreased rates throughout the 60 min operant session. However, during withdrawal responses were slightly depressed during the first half of the session, then decreased precipitously during the latter half.

On the eighth day these behavioral changes began to assume a very different pattern. During the eighth to ninth day after withdrawal a significant overshoot in performance

was seen suggesting the development of a possible protracted phase. Even though total responding during the entire hour session remained at control levels, performance during the first 30 min of the testing session remained elevated for at least 2 days. While the significance of this phenomenon is unknown, our data suggest that the behavioral consequences of clonidine withdrawal can persist for a long period. A more definitive description of this phase of the withdrawal syndrome is currently in progress.

Although clonidine can attenuate symptoms of withdrawal in morphine-dependent rats given naloxone, including a disruption of FR operant behavior, there was no reliable evidence that clonidine produced opiate-like tolerance-dependence. Naloxone, in doses capable of suppressing operant behavior, causing body weight loss and other symptoms of withdrawal in morphine-dependent rats [3] failed to disrupt the operant in clonidine-tolerant (dependent) rats. Body weight loss upon spontaneous withdrawal was not observed. Additionally, clonidine failed to alter binding of radio-labeled naloxone or etorphine to opiate receptors on guinea pig brain membranes (A. Goldstein, personal communication). In all likelihood, clonidine is producing an action upon pathways which subserve the expression of analgesia, tolerance and/or withdrawal in series or parallel to pathways containing the specific opiate receptors or at different receptors in the same pathway.

Withdrawal effects have been previously reported in humans [4] in which abrupt clonidine cessation caused a rebound overshoot in blood pressure. Whether such an effect may contribute to the disruption of behavior was not determined but will be pursued in future experiments.

ACKNOWLEDGEMENTS

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