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Associative Influences on Tolerance to Decreased Fixed-Interval Responding by Clonidine

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SMITH, J. B. *Associative influences on tolerance to decreased fixed-interval responding by clonidine*. PHARMACOL BIOCHEM BEHAV 36(4) 757–760, 1990.—Responding of rats was maintained under a 5-min fixed-interval schedule of food presentation. One group of animals (n = 5) received the alpha-2-agonist clonidine (0.1 mg/kg/day) *before* experimental sessions for 16 weeks. Additional animals (n = 5) also received 0.1 mg/kg/day for 16 weeks but experienced drug administration *after* sessions for 4 weeks, *before* sessions for 4 weeks, *after* sessions for 4 weeks, and then finally, *before* sessions for 4 weeks. Animals receiving clonidine *before* daily experimental sessions for the entire period developed tolerance to decreased responding within 3 weeks, and their responding remained near control levels except when clonidine was occasionally preceded by the alpha-2-antagonist yohimbine. Animals receiving clonidine *after* sessions did not develop tolerance, and responding was markedly suppressed during the first exposure to presession clonidine. When these animals subsequently received clonidine again *after* sessions, responding was disrupted (increased) in spite of continued drug administration as if animals were "dependent" on clonidine in specific circumstances. When these animals again received clonidine *before* sessions, responding was partially suppressed in spite of uninterrupted drug administration as if animals had "lost" tolerance in specific circumstances. Tolerance to the behavioral effects of clonidine on fixed-interval responding was not determined by the presence of drug alone, but by the associative influence of drug-related effects in the presence of specific environmental stimuli.

Clonidine Tolerance Fixed-interval responding

IT has become a useful procedure when studying behavioral influences on drug tolerance to compare effects of drugs when they are initially administered *after*, and then *before*, repeated experimental sessions. Since effects of postsession drug administration do not typically coincide with environmental stimuli associated with behavioral procedures, any tolerance that develops during these postsession drug administrations cannot involve behavioral processes associated with those procedures. A variety of experiments has used this "*before-after*" procedure and shown that tolerance to the behavioral effects of drugs does not always develop when chronic administration occurs outside the experimental environment, but rather is strongly influenced by both operant and respondent processes occurring in the presence of drug [e.g., (7, 14, 17–19)]. The purpose of the present experiment was to further study conditions in which the development of tolerance to effects of a drug on operant behavior can be influenced by the associative presence of specific environmental stimuli.

Responding was maintained under a fixed-interval schedule of food presentation and the alpha-2-adrenergic agonist clonidine was administered both *after* and *before* experimental sessions according to an ABAB within-subjects experimental design. A fixed-interval schedule was chosen because of its widespread use for studying behavioral effects of drugs. Clonidine was chosen because of its well-identified acute and chronic effects for both physiological (9,15) and operant responses (6, 8, 10, 13, 20), and because of its drug-sharing antinociceptive (2, 3, 11, 16, 21), hypotensive (5,9), and withdrawal-suppressing (4,22) effects with morphine. In addition, tolerance that develops to decreased motor (1) and operant (12) responding produced by morphine generalizes to similar effects of clonidine when animals are tested for cross-tolerance in the same environment in which they experienced the effects of morphine. Since clonidine is widely and chronically used in humans for its hypotensive and antiwithdrawal effects, and since it shares several effects with the narcotic morphine, it is important to try and fully characterize its broader effects on behaviors other than those that are clinically targeted.

METHOD

Animals

Ten experimentally naive male Charles River albino rats (F344) were approximately 120 days old at the beginning of the experiment. Water was continuously available in home cages and experimental chambers, and animals were maintained at approximately 300 g body weight with a diet of Noyes Pellets Formula A and Purina Rat Chow.

Apparatus and Behavioral Procedure

Experiments were conducted with individual rats placed in a ventilated, sound-isolated chamber. The inner cage was 23 cm long, 20 cm wide, and 20 cm deep, and contained a Gerbrands rat

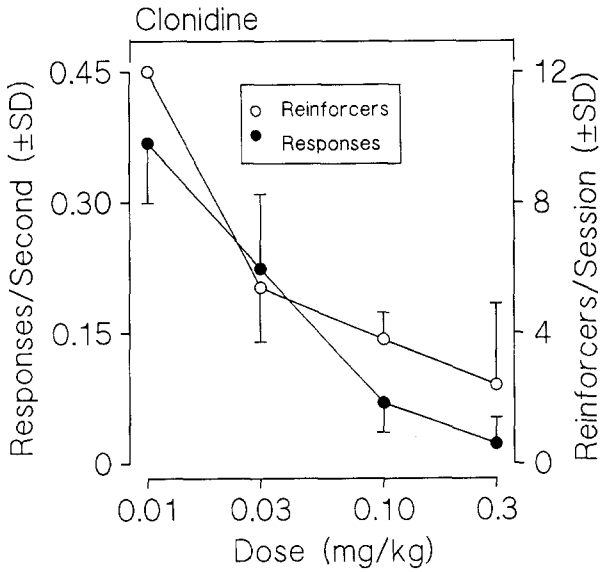

FIG. 1. Effects of acutely administered clonidine on responses per second (± 1 SD) and food reinforcers per 60-min session (± 1 SD; in parentheses) for rats (n = 10) responding under a 5-min fixed-interval schedule.

lever (centered), a food cup (adjacent to the left wall), and a Gerbrands response key (No. G6310, adjacent to the right wall), all mounted on the front panel. A recessed audio speaker and a water tube were mounted on the rear panel. All attached units were mounted 5 cm above the grid floor. The response key could be transilluminated by a white light, and responses were defined by an activation of the key with a minimum force of about 15 g. Responses on the lever had no programmed consequences. Reinforcers were three 0.045-g Noyes food pellets delivered by a solenoid-operated feeder. Programming and recording equipment were located in an adjacent room. Key pressing was trained by selectively reinforcing desired features of behavior, and responding was initially maintained under a 1-response fixed ratio schedule which delivered single food pellets in the presence of a white keylight. Subsequently, responding was maintained under a 5-min fixed-interval schedule in which the first key press occurring after 5 min in the presence of the white keylight resulted in three food pellets and a 5-sec period during which the white keylight was extinguished and responding has no programmed consequences. The timeout between fixed-interval periods was to enhance schedule control. If a response did not occur after 5.5 min in a fixed-interval period, that period terminated, the keylight was extinguished for 5 sec and the next interval began. Experimental sessions were conducted Monday–Friday and comprised 12 fixed-interval components (approximately 60 min). Animals responded under these conditions until variability of daily response rate was within 20% for two successive weeks.

Drug Procedure

Clonidine hydrochloride (courtesy of Boehringer-Ingelheim) and yohimbine hydrochloride (Sigma) were dissolved in 0.9% sodium chloride and injected IP in a volume of 0.5 ml/kg. Clonidine was administered either immediately *before* or immediately *after* experimental sessions or at noon on Saturdays and Sundays. Clonidine has a rapid onset of action and the immediate preinjection permitted observation of initial behavioral effects. When yohimbine was studied, it was administered 10 minutes prior to clonidine and animals were returned to their home cages during the wait. After initial training and development of stable performance (approximately 60 sessions of fixed-interval responding), all animals received at least 5 acute injections of several doses of clonidine (0.01–0.3 mg/kg). These injections occurred once weekly in a mixed order. Drug effects were determined for overall rate of responding (responses per second) and number of reinforcers per session.

After determining acute dose effects, animals were randomly divided into two groups of equal size. A control group received 0.1 mg/kg/day immediately prior to daily experimental sessions for 16 weeks. Under this condition, pharmacologic effects of clonidine were always coincident with behavioral processes in the experimental chamber. The remaining animals were designated the experimental group and received 0.1 mg/kg/day clonidine according to the following sequence: *after* sessions for 4 weeks; *before* sessions for 4 weeks; *after* sessions for 4 weeks; and *before* sessions for 4 weeks. Under this condition, pharmacologic effects of clonidine were coincident with both pertinent behavioral processes occurring in experimental chambers (the *before* sessions) and with unobserved processes occurring in individual home cages (the *after* sessions).

Control animals also received occasional "probe" injections of 1.0 mg/kg yohimbine as well as different doses of clonidine during chronic 0.1 mg/kg/day clonidine. These additional injections were for two purposes: to permit reassessment of clonidine dose effects after tolerance had developed and to determine the joint effects of chronic clonidine and a potent alpha-2 antagonist.

RESULTS

Control responding was characterized by a pause at the beginning of each fixed interval followed by relatively steady responding until the delivery of food. Overall responding for all 10 animals occurred at 0.28–0.46 responses/second (total range), and there were never instances when responding failed to produce food pellets by the end of the 5-min fixed-interval and 30-sec grace period. The mean and 99% confidence limits for control response rate were 0.38 ± 0.09 ($df = 9$).

When clonidine was given acutely, responding was systematically decreased as dose increased and 0.1 mg/kg markedly suppressed behavior and substantially reduced reinforcer frequency (Fig. 1). When animals in the control group received 0.1 mg/kg/day clonidine prior to repeated daily sessions, responding began to recover within a few days and was within control range within 3 weeks (Fig. 2A, open points). When experimental animals received the same dose immediately following experimental sessions, responding appeared normal during successive sessions, indicating that the duration of action of clonidine was short enough to preclude drug presence 24 hr after injection (Fig. 2A, filled points).

Responding of experimental animals was suppressed when 0.1 mg/kg clonidine was first administered *before* experimental sessions, but recovery occurred within 3 weeks (Fig. 2B, filled points). The rate of recovery for these animals did not appear any more rapid than for control animals even though these experimental animals had already received 4 weeks of daily clonidine after experimental sessions. When the drug was once again administered *after* sessions for experimental animals, responding increased moderately for several sessions, but recovery occurred within 1 week [Fig. 2C, filled points; effects during the first four sessions of Fig. 2C were significantly higher than those during the last four sessions of Fig. 2B, $t(4) = 6.78$, $p < 0.05$, but not significantly higher than those during the last four sessions of Fig. 2C, $t(4) = 2.53$]. When clonidine was once again injected *before* sessions for experimental animals, responding again decreased

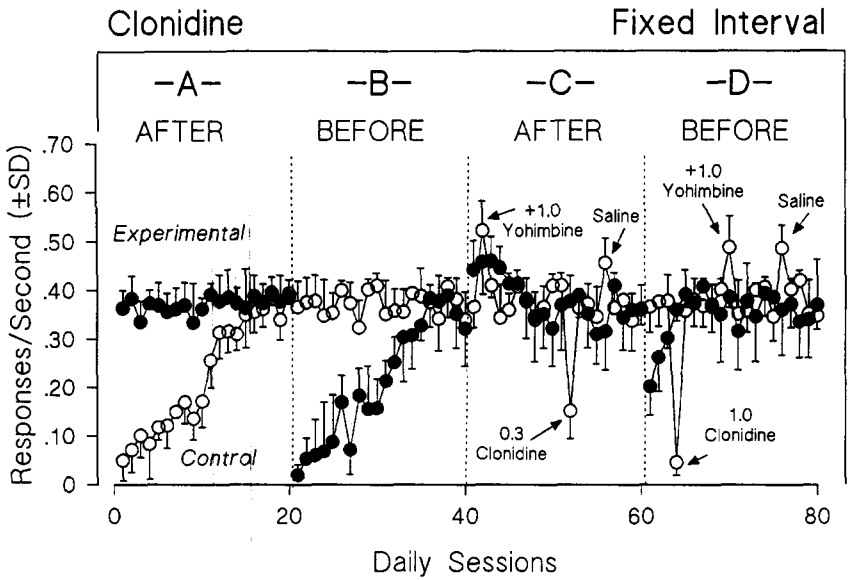

FIG. 2. Effects of daily administration of clonidine on responses per second (±1 SD) for rats responding under a 5-min fixed-interval schedule. Open points are for control animals (n=5) receiving clonidine *before* all sessions; filled points are for experimental animals (n=5) receiving clonidine either *before* (B and D) or *after* (A and C) sessions. Separately labelled points show effects of preceding clonidine with yohimbine or of different doses of clonidine.

below control level (Fig. 2D, filled points).

When control animals occasionally received 1.0 mg/kg yohimbine 10 min prior to daily clonidine, or received saline prior to sessions and 0.1 mg/kg clonidine after sessions (Fig. 2C and D, labelled open points), the regular pattern of responding was disrupted and rate of responding was outside the upper 99% confidence limit of responding during all nonprobe sessions shown in Fig. 2B–D (n=56). In addition, when animals in the control group occasionally received larger doses of clonidine, the dose-response curve was shifted to the right, so that responding was totally suppressed at 1.0 instead of 0.3 mg/kg (Fig. 2C and D, labelled open points).

DISCUSSION

Clonidine decreased key pressing of rats maintained under a 5-min fixed-interval schedule of food presentation, and tolerance developed within 3 weeks when drug administration preceded daily experimental sessions. These acute effects are consistent with previous reports that clonidine decreases fixed-interval responding in rodent (6), pigeon (6), and primate (11).

Even though tolerance developed for control animals receiving presession clonidine, tolerance did not initially develop for behavioral effects in experimental animals during 4 weeks of postsession clonidine. This was indicated by markedly reduced responding for experimental animals during the first several sessions of presession drug administration. Because tolerance developed to behavioral effects of clonidine only when the drug experience occurred during experimental sessions, this tolerance did not depend on pharmacologic processes alone, but also on behavioral processes associated with environmental stimuli in experimental chambers.

When clonidine was injected prior to sessions for experimental animals, tolerance developed to decreased responding at approximately the same rate it had for control animals. Consequently, previous administration of clonidine outside experimental sessions did not markedly influence the eventual rate of tolerance development when animals did begin receiving clonidine prior to experimental sessions.

When clonidine was once again administered *after* sessions for the experimental group, responding was increased for several sessions. It is often described that behavioral disruptions produced by withholding of chronic drug reflects drug dependence. The present results suggest a similar "withdrawal" for clonidine even in the continued presence of drug. Instead of completely removing drug-produced stimuli, however, the present procedure removed drug stimuli from the total stimulus complex associated with tolerance development while continuing the physiological presence of drug. It is noteworthy that removing drug-produced stimuli during postsession clonidine with experimental animals had similar effects as preceding chronic clonidine with the alpha-2-adrenergic antagonist yohimbine and with substituting saline for clonidine with control animals. In each of these instances, responding of drug-tolerant animals was disrupted (increased) when clonidine was absent from its pharmacologic site of action.

When clonidine was again administered prior to daily sessions for experimental animals following a period of postsession drug administration, fixed-interval responding was once again decreased. Thus, even though animals had continued to receive the same daily dose of clonidine (now in the 16th week), there was a partial "loss" of tolerance when drug administration began occurring after sessions and apart from pertinent behavioral processes.

The joint effects of yohimbine and clonidine, as well as the dose effects of clonidine during chronic dosing and the effects of substituting saline for clonidine, all provided clear evidence that clonidine was pharmacologically active (presumably at alpha-2-adrenergic receptors) during all phases of the present experiment. However, the manipulations of concurrence between drug administration and performance of fixed-interval responding provided equally clear evidence that tolerance to the *behavioral* effects of clonidine was markedly influenced by the joint occurrence of pharmacologic and pertinent behavioral processes. Ongoing experiments are studying the extent to which tolerance

which develops for a particular schedule-controlled performance in a particular experimental chamber generalizes to both the same and to different operant performances in different environmental circumstances. These results can indicate the extent of associative influences on tolerance to operant performance.

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