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Tolerance to Behavioral Effects of Clonidine After Chronic Administration of Morphine

JAMES W. McKEARNEY

Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

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McKEARNEY, J. W. *Tolerance to behavioral effects of clonidine after chronic administration of morphine.* PHARMACOL BIOCHEM BEHAV 22(4) 573–576, 1985.—Male rats (Buffalo strain) were studied under a procedure in which each 30th lick of a drinking tube resulted in the delivery of 0.01 ml water. The effects of clonidine HCl (0.003–0.3 mg/kg, IP) were determined before, during and after exposure to conditions in which a morphine sulfate solution (0.5 mg/ml in 0.4% saccharin) was the only source of fluid. After either 10 or 80 days exposure to the chronic morphine regimen, rats were maintained under a repetitive cycle in which the morphine was available for 3 days and then removed for 4 days. The subjects consumed an average of 100 mg/kg/day morphine during the times it was available. The effects of clonidine were redetermined once weekly, on the 4th day after removal of the morphine solution. The effects of clonidine were also determined after morphine was removed for more prolonged periods (18–67 days). Chronic exposure to the morphine solution resulted in a 4- to 5-fold shift to the right in the dose-effect curve for clonidine (decreased responding). ED50 values returned to pre-morphine levels when rats were tested at longer post-morphine times (e.g., 18 days). Under the conditions of this experiment, chronic exposure to morphine produced marked cross-tolerance to the behavioral effects of clonidine.

Clonidine Morphine Cross-tolerance Fixed-ratio schedules Rats

MANY of the behavioral effects of clonidine and morphine are similar. For example, both suppress locomotor activity and operant behavior, have antinociceptive effects under a variety of procedures, and are self-administered by experimental animals (for a review, see [6]). Further, it is widely reported that clonidine can alleviate withdrawal signs seen after cessation of chronic opioid treatment in animals and humans [1, 14, 15]. However, the mechanisms of action underlying these effects are at least partially distinct. The behavioral effects of acutely administered morphine are blocked by opioid antagonists such as naloxone and not by α_2-adrenoreceptor antagonists such as yohimbine, whereas the reverse pattern of antagonism is seen with clonidine [4,6].

There is marked tolerance development to the various behavioral actions of morphine, but tolerance to the effects of clonidine is not always seen. There are reports of tolerance to the effects of clonidine on locomotor activity [7] and on suppression of responding under a fixed-ratio schedule of food presentation [8]. However, there is another report of no apparent tolerance to the suppressing effect of clonidine given over a 30-day period (0.1 mg/kg/day) in rats responding under a continuous shock-avoidance schedule [11]. There are also mixed reports on tolerance to the antinociceptive effects of clonidine. Paalzow [9] reported tolerance to the effects of clonidine on shock-induced vocalization (increasing doses twice daily over a 7-day period). On the other hand, there was no tolerance to the effects of chronically administered clonidine under the mouse tail-flick or rat tail-withdrawal procedures [3], and only partial tolerance in squirrel monkeys studied under an electric shock titration schedule [12]. In view of the diversity of species, test procedures, and dosing regimens studied in these experiments, it is not possible to specify the conditions under which tolerance to the effects of clonidine may be observed.

It is likewise unclear whether there is reliable cross-tolerance between the effects of morphine and clonidine. Paalzow [9] did observe tolerance to the effects of morphine in clonidine-tolerant rats studied under a shock-induced vocalization procedure, but other investigators [13] did not observe tolerance to the effects of clonidine in morphine-tolerant mice studied under the tail-flick procedure. This difference may indicate that cross-tolerance is not reciprocal, but differences in species and test procedures may be responsible. There seem to be no published reports regarding possible changes in the effects of clonidine on operant behavior in subjects chronically treated with morphine. Accordingly, the present experiments determined the effects of clonidine on fixed-ratio responding before, during, and after continuous exposure to morphine.

METHOD

Animals

Five male rats of the Buffalo strain (Harlan Sprague Dawley, Inc.) were approximately 60 days old at the beginning of training and 90 days old at the beginning of drug testing. The experiments were conducted over about a 1-year period after initial training. Food was freely available in home cages

throughout the experiments. Water was available from just after experimental sessions on Fridays until Monday a.m. At other times, access to water was limited to 30 min after daily experimental sessions. Subjects weighed an average of 249 grams (range of 235–275) at the beginning of drug testing, and 359 grams (range of 345–408) at the conclusion of experiments.

Apparatus and Behavioral Procedure

Experiments were conducted in a rectangular Plexiglas chamber (12×22×20 cm high), housed in a sound-attenuating enclosure. A small circular opening 3 cm up from the center of the grid floor on one wall allowed tongue access to the tip of a solenoid-operated water dispenser (Coulbourn Electronics No. E14-03, free-flow tip). When activated, this device delivered approximately 0.01 ml of water per pulse.

Subjects were deprived of water and placed in the chamber, and all made regular contact with the drinking device within the first experimental session. Then, the response requirement was gradually increased so that 30 responses (licks, sensed by a low current contact-sensor) were necessary for water delivery. Each water pulse (0.01 ml) was followed by a 1.5-sec period in which licks were not counted toward the response requirement (the presumed period of consummatory licking). Experimental sessions were 30 min in duration, conducted 5 days weekly.

Drug Procedure

Clonidine HCl (courtesy of Boehringer Ingelheim) was dissolved in sterile distilled water and injected IP in a volume of 1.0 ml/kg immediately before experimental sessions. During certain phases of the experiments (Table 1), subjects were given continuous access to a solution of 0.5 mg/ml morphine sulfate (Merck) in 0.4% saccharine (w/v in tap water) as their only source of fluid (conditions B and D, Table 1). No behavioral testing was done during these periods of continuous access. In experimental phases when the effects of clonidine were tested during chronic morphine exposure, the morphine solution was available from just after experimental sessions on Fridays until Monday at about 10 a.m. Subjects were given 30-min access to tap water after experimental sessions on Tuesday through Thursday, and the effects of morphine were determined on Friday (about 96 hr after removal of the morphine solution). Clonidine was given no more than once weekly.

Drug effects are expressed in terms of response rate as a percent of the rate observed during non-drug control sessions (usually Thursdays). Estimated doses producing a 50% reduction in responding (ED50 and 95% confidence limits) were calculated from least-squares lines fitted to log dose-response curves [5]. Since dose-effect curves were relatively linear, effects were not transformed to probits. Paired-comparison t-tests were also performed using ED50 values obtained from individual subjects.

TABLE 1

SEQUENCE OF EXPERIMENTAL CONDITIONS

A. Clonidine administration prior to exposure to chronic morphine [ended 19 Aug 1983].
B. 80 days of continuous morphine availability, then redetermination of clonidine effects at 96 hr after morphine removal (morphine available for 72 hr prior to each 96-hr test period [ended 02 Dec 1983].
C. Redetermination of clonidine effects beginning at 28 days after cessation of morphine availability [ended 19 Jan 1984].
D. 10 days of continuous morphine availability, then redetermination of clonidine effects at 96 hr (as in B) [ended 09 Mar 1984].
E. Redetermination of clonidine effects beginning at 18 days after cessation of morphine availability [ended 13 Apr 1984].
F. Redetermination of clonidine effects beginning at 60 days after cessation of morphine availability [ended 11 May 1984].

RESULTS

The fixed-ratio schedule of water presentation engendered a high and steady rate of responding in all subjects (about 3 responses/second over the 30-min experimental sessions).

Table 2 summarizes the intake of morphine in individual subjects during the various experimental conditions. Subjects consumed an average of approximately 100 mg/kg/day morphine over the periods in which it was available in the drinking water (conditions B and D, Table 1). Although there was inter-subject variabiltiy in the amount of morphine consumed, consumption within individual subjects was relatively constant over the course of the experiments. When tested under the FR schedule at various times after removal of morphine, responding was markedly suppressed 24 hr later and gradually recovered to control levels by 72 hr. Therefore, the effects of clonidine were assessed on the 4th day (≈96 hr) after removal of the morphine solution (with 30-min access to tap water on intervening days).

Figure 1 summarizes the effects of clonidine on responding during the various phases of the experiments. Either prior to or after 28+ days absence of morphine, the ED50 dose was about 0.02 mg/kg clonidine (unfilled circles, pooled data from conditions A and C). When tested during the first (B) or second (D) period of exposure to chronic morphine, however, the dose-effect curve was shifted about 4- to 5-fold to the right (filled circles, ED50s of 0.096 and 0.078 respectively for conditions B and D). When again retested after prolonged morphine absence (18+ days), the effects of clonidine were similar to those seen earlier (unconnected triangles). There were no further changes in the effects of clonidine when rats were again tested at 60+ days after morphine removal (unconnected open circles).

Figure 2 shows ED50 values and 95% confidence limits (two-tailed t-distribution) for clonidine during the various experimental conditions (based on pooled data for all subjects). ED50 doses were markedly higher when subjects were tested during either of the periods of chronic morphine treatment (B and D) than they were either before exposure to morphine (A) or during periods when morphine treatment had been suspended for prolonged periods (C and D). Paired-comparison t-tests of ED50 values were significantly higher during phases B or D than during either A, C, or E ($p<0.02$), whereas values during morphine-free conditions (A, C, E) did not differ significantly ($p>0.05$).

DISCUSSION

Chronic consumption of morphine resulted in significant and reliable tolerance to the response-decreasing effects of clonidine under the fixed-ratio schedule of water presentation. Although the minimum period of morphine exposure necessary for this change was not determined, there was a clear shift under the condition in which a 10-day period of continuous morphine availabiltiy was followed by a regimen

TABLE 2

AVERAGE MORPHINE INTAKE IN VARIOUS PHASES (mg/kg/day, 2 SE IN PARENTHESES)

Rat No.	80-Day Continuous	First Clonidine Test	10-Day Continuous	Second Clonidine Test
1	102 (9)	103 (17)	76 (38)	94 (11)
2	76 (12)	78 (22)	60 (18)	78 (16)
10	104 (14)	138 (17)	145 (51)	117 (13)
14	111 (14)	105 (27)	100 (33)	106 (24)
20	94 (14)	117 (13)	90 (36)	102 (26)

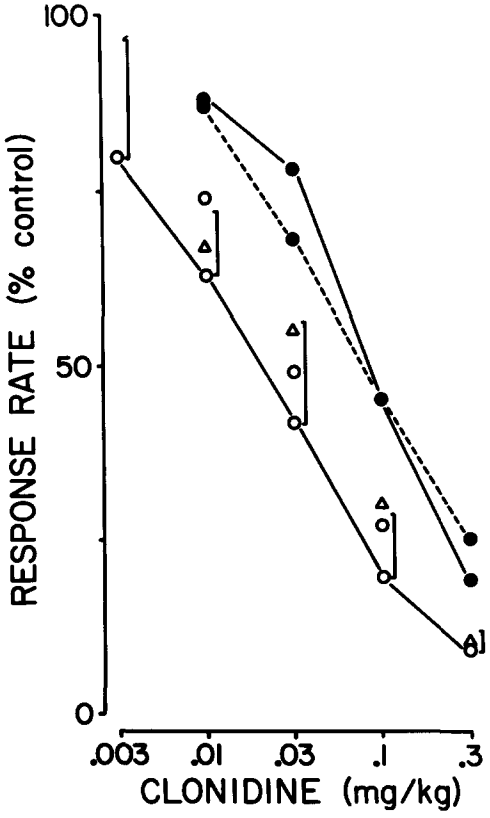

FIG. 1. Effects of clonidine on fixed-ratio responding before, during, and after chronic exposure to morphine. ○—○: pooled data from pre-morphine and 28+ days post-morphine (conditions A and C, brackets are 95% confidence limits). Filled circles: 96 hr after morphine removal during first (●—●) or second (●— —●) period of chronic morphine availability (conditions B and D, respectively). Unconnected triangles show effects at 18+ days after termination of the second period of chronic morphine (condition E), and unconnected circles show effects during the final condition (F). In general, points are averages of no less than 5 observations.

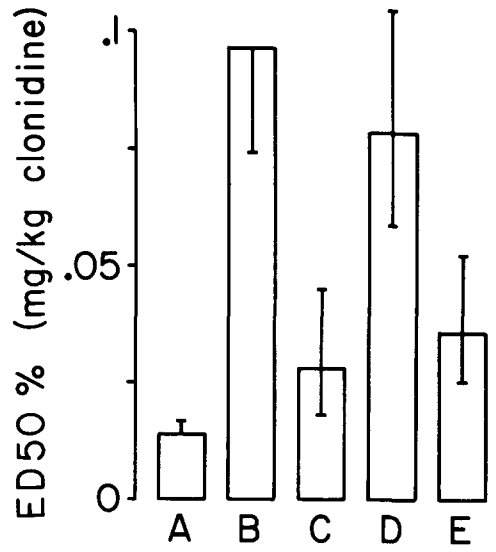

FIG. 2. Clonidine ED50 values during various experimental conditions (pooled data for all subjects). Vertical lines indicate 95% confidence limits (two-tailed t-distribution). See Table 1 for explanation of experimental conditions. ED50 values were calculated by standard methods [5] without transformation. ED50 values and 95% confidence limits for the various conditions were as follows: A, 0.014 (0.011–0.017); B, 0.096 (0.074–0.126); C, 0.029 (0.018–0.045); D, 0.078 (0.058–0.104); E, 0.036 (0.025–0.052). The slopes of the regression lines did not differ significantly ($p>0.10$ for all comparisons).

in which morphine was available only for 3 days weekly. Although the degree of tolerance to the effects of morphine itself was not determined here, the magnitude of the average amount consumed (≈100 mg/kg/day) makes it likely that marked morphine tolerance did develop.

Further work will be necessary to determine the generality of the type of cross-tolerance seen here, and whether or not it is reciprocal in nature. Experiments with other rat strains and other species are also needed, since there have been reports [2,10] of differences in ^{3}H-clonidine binding in certain brain areas in rats of the Buffalo strain (used here) as compared with those of the Fisher F-344 strain.

Along with the many demonstrations of commonalities in the behavioral effects of morphine and clonidine, and of the ability of clonidine to counteract morphine-withdrawal symptoms, the present and earlier [9] observations of cross-tolerance support the view that certain of the behavioral effects of the two drugs may be mediated by some common final mechanism.

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