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Blocking Naloxone-Precipitated Withdrawal in Rats and Hamsters

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SCHNUR, P., M. ESPINOZA, R. FLORES, S. ORTIZ, S. VALLEJOS AND M. WAINWRIGHT. *Blocking naloxone-precipitated withdrawal in rats and hamsters.* PHARMACOL BIOCHEM BEHAV 43(4) 1093–1098, 1992.—Three experiments studied the effects of putative antagonists of opiate withdrawal in hamsters and rats. In Experiment 1, the calcium channel antagonists verapamil (20 mg/kg) and nifedipine (20 mg/kg) failed to antagonize naloxone (1 mg/kg)-precipitated withdrawal in hamsters implanted with two 75-mg morphine pellets, whereas clonidine (0.4 mg/kg), the α_2-adrenergic agonist, blocked most withdrawal signs. In Experiment 2, clonidine (0.4 mg/kg) and verapamil (20 mg/kg) were tested against naloxone-precipitated withdrawal in hamsters made acutely dependent by a single injection of morphine (15 mg/kg). As in Experiment 1, clonidine but not verapamil was effective. In Experiment 3, the effects of verapamil on naloxone-precipitated withdrawal were studied in morphine-pelleted rats and hamsters. In rats implanted with two morphine pellets, verapamil (20 mg/kg) reversed naloxone-precipitated withdrawal. By contrast, in hamsters implanted with either one or two morphine pellets neither of two doses of verapamil (20 and 30 mg/kg) was effective. These results are discussed in terms of species' differences in sensitivity to calcium channel blockers.

Calcium channels Opiate dependence Acute dependence Verapamil Nifedipine Clonidine Morphine Naloxone Precipitated withdrawal Rats Hamsters

PHARMACOTHERAPY for opiate abuse is directed at easing the discomfort associated with the abstinence syndrome. One pharmacotherapeutic approach is to treat the withdrawal symptoms elicited by abstinence with an opiate such as methadone or with its longer-acting congener, levo-α-acetylmethadol [(LAAM); (22)]. Although opiates block withdrawal effectively, they do so by maintaining the physically dependent state and they have potential for abuse, as well. An alternative approach is to treat withdrawal symptoms with a nonopiate, such as the α_2-adrenergic agonist clonidine (17). Although clonidine does not maintain physical dependence and has no abuse potential, its use is limited by variable efficacy in different individuals and, at high doses, by side effects such as hypotension and sedation. Thus, a need exists for additional pharmacotherapeutic agents that would be useful in the treatment of opiate abuse. The experiments conducted below were designed to test the effects of calcium channel antagonists on naloxone-precipitated withdrawal in opiate-dependent hamsters and rats.

Accumulating evidence indicates that opiate tolerance and dependence are correlated with neuronal calcium levels and that calcium channel agonists and antagonists have modulatory influences on the behavioral and physiological responses to opiates. For example, in mice calcium uptake increases following morphine pellet implantation (18), the increase is proportional to the duration of pellet implantation (18), and it declines to baseline levels during naloxone-precipitated withdrawal (20). In addition, whereas manipulations that increase neuronal calcium levels attenuate the analgesic effects of morphine and μ-opioids (13,19,24) manipulations that decrease neuronal calcium potentiate the analgesic and hypothermic effects of morphine and μ-opioids (8,15,19,20,24,26). Further, calcium channel antagonists block the opiate withdrawal syndrome in both in vitro and in vivo systems (2–7,9,12,28, 31,38). Finally, the density of calcium channel binding sites increases in animals treated chronically with morphine (27, 28,42).

The present study was designed to test the effects of calcium channel antagonists on naloxone-precipitated withdrawal in morphine-dependent hamsters. Because the relative distribution of opioid receptor subtypes in the human brain is more similar to that in the hamster than in the rat (29,41), an understanding of opiate effects and opiate dependence in the hamster might prove especially important. Previous research in our laboratory demonstrated that opiate dependence develops readily in the hamster and naloxone-precipitated withdrawal occurs reliably following acute and chronic exposure to morphine (32,33). To date, however, there have been no tests of the effects of calcium channel antagonists in the opiate-dependent hamster. Thus, the effect of calcium channel

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antagonists on naloxone-precipitated withdrawal was tested in both acutely and chronically treated hamsters. Similarly, the effect of clonidine has not been investigated in opiate-dependent hamsters. Because clonidine has been shown to inhibit opiate withdrawal in a variety of in vivo and in vitro tests (1,10,11,14,17,25,30,35-38), a demonstration of its efficacy here would increase the validity of the hamster model of opiate dependence. Moreover, the reported efficacy of clonidine against naloxone-precipitated withdrawal is equivalent to that of calcium channel blockers in several in vitro and in vivo systems (2,3,28). Thus, the present study compared the effects of calcium channel antagonists with clonidine on precipitated withdrawal. Finally, because calcium channel antagonists have been reported to block opiate withdrawal in rats the present work compared the effect of calcium channel antagonists on precipitated withdrawal in rats and hamsters.

GENERAL METHOD

SUBJECTS

Golden Syrian hamsters weighing approximately 120 g and Sprague-Dawley rats weighing approximately 285 g were used. Rats and hamsters were housed in separate rooms in a temperature-controlled vivarium in hanging stainless steel cages with free access to food and water. All experiments were conducted in accordance with NIH guidelines for the use and care of laboratory animals.

APPARATUS AND MATERIALS

Animals were observed for symptoms of withdrawal in a transparent polycarbonate cage (45.7 × 24.1 × 20.3 cm). The following drugs (and doses) were used: morphine sulfate (15 mg/kg), morphine sulfate (75-mg pellets), naloxone HCl (1 mg/kg), clonidine HCl (0.4 mg/kg), verapamil HCl (20 mg/kg), and nifedipine (20 mg/kg). Morphine, naloxone, verapamil, and clonidine were dissolved in saline vehicle and injected in 1-ml/kg volumes. Nifedipine was dissolved in a 1% Tween-80 in saline vehicle and also administered in 1-ml/kg volumes.

BEHAVIORAL MEASURES

Animals were tested individually by observers who were blind as to group assignment. Interrater reliability estimates for several behavioral measures of withdrawal ranged between 0.82–0.93. Behavior was sampled continuously during the 40-min observation periods throughout the experiment. Signs of withdrawal including paw tremors, wet-dog shakes, abdominal writhing, teeth-chattering, and yawning were counted. Paw tremors refer to vigorous shaking of the front or rear paws that is unrelated to grooming or scratching. Wet-dog shakes refer to torsional shakes involving the head and shoulders. Abdominal writhing was noted when the animal rotated its torso while pressing its abdomen to the floor, typically accompanied by arching of the back. Teeth-chattering refers to tremors in the jaw muscles that produce visible movements of the mouth and muscles of the face, often accompanied by audible knocking of the teeth. Teeth-chattering occurs in bouts of approximately 2- to 3-min duration. Within a bout, continuous chatters of approximately 6- to 8-s duration alternate with quiescent periods of approximately 5 s. The frequency of teeth-chattering refers to the number of continuous chatters during the observation period. Yawning needs no explanation.

DATA ANALYSIS

A composite withdrawal score (the sum of paw tremors, wet-dog shakes, abdominal writhing, teeth-chattering, yawning) was calculated for each animal. Data were analyzed initially in terms of the composite score and subsequent analyses of the individual responses were conducted. A significance level of $p < 0.05$ was adopted throughout.

EXPERIMENT 1

The purpose of Experiment 1 was to test the effects of verapamil, nifedipine, and clonidine on naloxone-precipitated withdrawal in morphine-dependent hamsters. Drug doses were based upon previously reported findings in rats and mice (7,9,37) and pilot observations in the hamster in our laboratory.

METHOD

Sixteen hamsters were implanted SC with two morphine pellets under halothane anesthesia. On postimplant days 1, 2, 3, 5, and 7, four randomly constituted groups ($n = 4$) received an IP injection of either vehicle, clonidine, verapamil, or nifedipine immediately before being placed in the plastic observation cage for 40 min. Following a 10-min baseline period of observation, all animals received an SC injection of naloxone (1 mg/kg) and were replaced in the plastic cage for an additional 30-min period of observation.

RESULTS

Clonidine, but neither verapamil nor nifedipine, inhibited naloxone-precipitated withdrawal. Moreover, the effect of clonidine was selective, inhibiting wet-dog shakes, teeth-chattering, and yawning but not paw tremors. The frequency of naloxone-precipitated abdominal writhing in all groups was too low to permit meaningful comparisons. The effect of clonidine, verapamil, and nifedipine on naloxone-precipitated wet-dog shakes, teeth-chattering, and yawning is shown in Fig. 1 as a function of postimplant days. It can be seen that, compared with saline controls, only clonidine suppressed these withdrawal symptoms and the effect was evident on each postimplant day. Separate four (drug) × five (days) mixed-factorial analyses of variance (ANOVAs) indicated that clonidine suppressed wet-dog shakes, $F(3, 12) = 7.62$, teeth-chattering, $F(3, 12) = 14.47$, and yawning, $F(3, 12) = 4.51$. For each of these responses, post hoc analyses using Fisher's least significant differences (LSD) test indicated that animals given clonidine exhibited fewer responses than each of the other groups, which did not differ among themselves. It is possible that the failure of the calcium channel antagonists to block naloxone-precipitated withdrawal was due to the high level of dependence achieved in the morphine-pellet-implanted hamster. Nevertheless, at the doses tested clonidine proved to be the more effective antagonist of opiate withdrawal in the hamster.

EXPERIMENT 2

The purpose of the second experiment was to test the effects of verapamil and clonidine on naloxone-precipitated withdrawal in hamsters treated acutely with morphine. It was reasoned that the effects of verapamil might be more readily detected in the acutely treated animal than in the chronically treated animal used in Experiment 1. Moreover, the effect of clonidine in acutely treated animals has not been previously investigated.

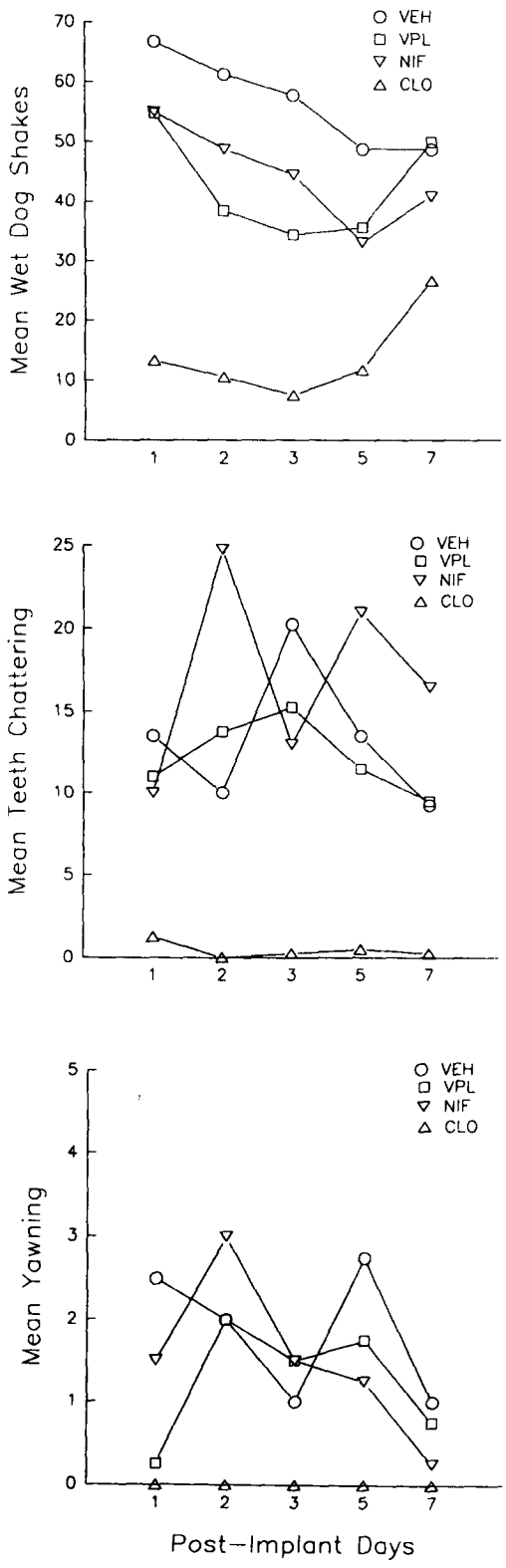

FIG. 1. Mean wet-dog shakes (top), mean teeth-chattering (middle), and mean yawning (bottom) as a function of postimplant days for each group in Experiment 1.

METHOD

The procedures of the second experiment were carried out in a single day. Twenty hamsters were assigned randomly to one of four groups (n = 5) that received a series of three injections. The first injection was given in the home cage. Forty minutes later, animals were given a second injection in the home cage. Twenty minutes later, animals were removed from the home cage and placed in the plastic observation cage. After a 10-min period of baseline observations, animals were injected SC with naloxone (1 mg/kg) and replaced in the plastic cage for an additional 30 min of observation. Group M/C received an initial SC injection of morphine (15 mg/kg) followed by an IP injection of clonidine (0.4 mg/kg). Group M/V received an SC injection of morphine followed by an IP injection of verapamil (20 mg/kg). Group M/S received an SC injection of morphine followed by an IP injection of saline. Group S/S received an SC injection of saline followed by an IP injection of saline. To reiterate, all groups then received an SC injection of naloxone 10 min after being placed in the plastic cage.

RESULTS

Figure 2 shows the mean number of withdrawal responses (composite score) elicited by naloxone in each group in Experiment 2. It is evident that the procedures of this experiment were sufficient to induce morphine dependence [cf. (32)], that is, a naloxone challenge potently precipitated withdrawal in animals given a single injection of morphine (group M/S) but not in opiate-naive controls (group S/S). It is evident also that clonidine but not verapamil effectively antagonized naloxone-precipitated withdrawal: Group M/C gave fewer withdrawal responses than group M/S, whereas group M/V gave as many withdrawal responses as group M/S. A one-way ANOVA indicated that the effect of drug was significant, $F(3, 16) = 4.76$. Post hoc analyses using Fisher's LSD test indicated that

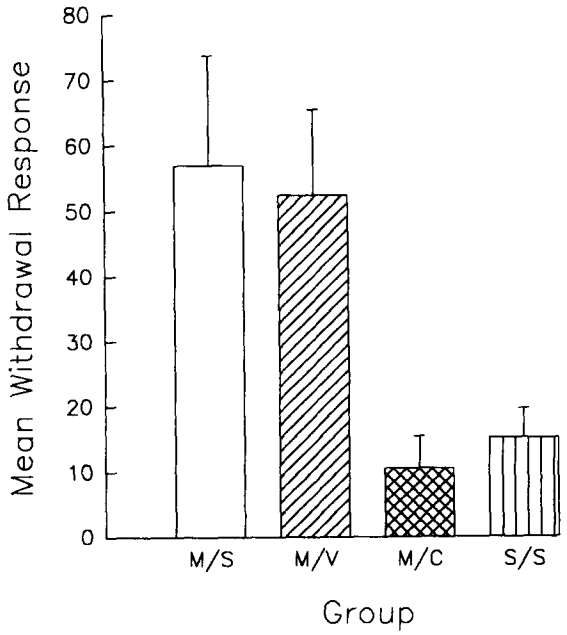

FIG. 2. Mean composite withdrawal response for each group in Experiment 2.

groups M/S and M/V each gave more withdrawal responses than either groups M/C or S/S, but that the groups within each of the preceding pairs did not differ from one another. Thus, as in Experiment 1, clonidine but not verapamil effectively antagonized naloxone-precipitated withdrawal in the hamster. In addition, clonidine's effect was response selective, as in Experiment 1. Separate analyses indicted that the drug effect on teeth-chattering, $F(3, 16) = 5.16$, and wet-dog shakes, $F(3, 16) = 3.66$, were significant, whereas the effect on paw tremors was not, $F(3, 16) = 2.88$.

EXPERIMENT 3

The failure of the calcium channel antagonists to block naloxone-precipitated withdrawal in Experiments 1 and 2 suggests that the effects of calcium channel antagonists might be different in hamsters as compared to rats and mice (7,9). Alternatively, it may be that the effective dose of verapamil is higher in the hamster than in other species. Experiment 3 was designed to investigate these hypotheses by directly comparing the effects of verapamil on naloxone-precipitated withdrawal in rats and hamsters. In Experiment 3a, the effect of verapamil (20 mg/kg) was tested in rats made dependent by the implantation of two 75-mg morphine pellets. In Experiment 3b, the effect of that same dose of verapamil was tested in hamsters made similarly dependent by the implantation of a single morphine pellet. The rationale for implanting two pellets in rats and one in hamsters was to equate dosage by body weight in the two species. In Experiment 3c, the effect of a 30-mg/kg dose of verapamil was tested in hamsters. Pilot observations indicated that doses of verapamil higher than 30 mg/kg (e.g., 40 mg/kg) are lethal in the hamster.

METHOD

Experiment 3a

Two days after implantation of two 75-mg morphine pellets, half the rats were injected IP with saline ($n = 4$) and half with verapamil (20 mg/kg; $n = 4$) 20 min prior to an IP injection of naloxone (1 mg/kg). Immediately after naloxone injection, animals were placed in the plastic cages for a 40-min observation period.

Experiments 3b and 3c

Two days after implantation of one 75-mg morphine pellet, half the hamsters were injected IP with saline ($n = 4$) and half with verapamil ($n = 4$) 20 min prior to an IP injection of naloxone. They were then placed in the plastic cages for a 40-min observation period. In Experiment 3b, the dose of verapamil was 20 mg/kg; in Experiment 3c, it was 30 mg/kg.

RESULTS

Verapamil blocked naloxone-precipitated withdrawal in rats but not in hamsters. These findings thus replicate previous research using rats (9), as well as the results of the first two experiments above using hamsters. A t-test indicated that the difference in the number of withdrawal responses between rats given verapamil (mean = 29) and those given saline (mean = 113) was significant ($t = 3.49$). In hamsters, there was no difference in the number of withdrawal responses between animals given saline (mean = 113) and those given 20 mg/kg verapamil (mean = 87), nor between those given saline (mean = 101) and those given 30 mg/kg verapamil (mean = 109).

GENERAL DISCUSSION

The principal finding in the present study was that the calcium channel blocker verapamil effectively antagonized naloxone-precipitated withdrawal in the rat [cf. (9,28)] but not in the hamster. Although it is possible that a different dosing regimen from that used here would have revealed an effect of verapamil in the hamster, we doubt it. In separate experiments, neither of two calcium channel antagonists showed reliable effects in the hamster. In Experiment 1, neither verapamil nor nifedipine blocked withdrawal whereas clonidine effectively antagonized several withdrawal signs in the morphine-pelleted hamster. In Experiment 2, the results were similar in the acutely dependent hamster. Thus, it is unlikely that verapamil's failure to block withdrawal in Experiment 1 was due to excessive levels of dependence produced by two implanted morphine pellets. In Experiment 3, verapamil again failed to reverse withdrawal in two separate experiments using high doses of verapamil and both one- and two-pellets implants. As noted above, doses of verapamil higher than 30 mg/kg proved lethal in pilot experiments with hamsters. In mice, by contrast, verapamil doses of 40 and 80 mg/kg are required to reliably reduce withdrawal symptoms (7).

The present results encourage the hypothesis that dependent hamsters are different from rats and mice in their response to verapamil, although an explanation for this species difference is not at hand. One possibility is that systemically administered verapamil does not reach the hamster brain either because it is completely metabolized in the periphery or because it does not penetrate the blood-brain barrier in the hamster. To test this hypothesis, eight hamsters were implanted with ICV cannulae aimed at the left lateral ventricle. On 4 test days, half the animals received an injection of morphine (15 mg/kg) followed 1 h later by naloxone (1 mg/kg) and half received saline followed by naloxone. In both groups, naloxone injection was preceded by an ICV infusion of one of four doses of verapamil (0, 1, 3, or 10 μg dissolved in 1 μg Ringer's solution). Animals then were observed for signs of opiate withdrawal. Although naloxone precipitated withdrawal in morphine-treated animals compared to saline controls, verapamil was ineffective in attenuating that withdrawal. The mean withdrawal scores for the morphine groups receiving 0, 1, 3, or 10 μg of verapamil were 41, 29, 61, and 54 respectively, scores that did not differ significantly.

The failure of verapamil and nifedipine to reverse precipitated withdrawal in hamsters suggests that caution be exercised in generalizing from studies reporting that calcium channel blockers antagonize withdrawal symptoms in rats and mice [e.g. (7,9)]. The existence of a difference in sensitivity to the effects of verapamil between rats and hamsters indicates that effects documented in one species may not be generalizable to other species. This caution is especially prudent when testing potential pharmacotherapeutic agents for use in human opiate abusers. Indeed, it has been reported recently that verapamil is without effect on subjective, behavioral, or physiological measures of naloxone-precipitated opiate withdrawal in humans (34).

The effects of clonidine on naloxone-precipitated withdrawal in hamsters are demonstrated in Experiments 1 and 2. Clonidine at a dose of 0.4 mg/kg antagonized withdrawal in both chronically and acutely dependent hamsters. Further, in

both cases the pattern of clonidine's effects on specific responses was similar. In Experiment 1, clonidine reversed the naloxone-precipitated wet-dog shakes, teeth-chattering, and yawning in morphine-pelleted hamsters but had no effect on paw tremors. In Experiment 2, clonidine reversed wet-dog shakes and teeth-chattering but had a small, nonreliable effect on paw tremors. These results are consistent with numerous studies indicating that clonidine acts selectively in rats and mice to reverse some withdrawal signs but not others (10, 11,16,23,37,39,40). Because clonidine's effects were similar in chronically and acutely treated hamsters, it might be suggested that a common mechanism underlies chronic and acute opiate dependence. Insofar as the inhibition of withdrawal signs by clonidine following chronic opiate treatment is attributable to clonidine's suppression of noradrenergic hyperactivity (1, 30,35,37), the present results suggest the hypothesis that acute opiate treatment also leads to noradrenergic hyperactivity.

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