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THE SUPPRESSION OF DEPRIVATION AND ANTAGONIST-INDUCED WITHDRAWAL IN MORPHINE-DEPENDENT RHESUS MONKEYS

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The capacity of morphine to suppress natural and precipitated withdrawal was compared in morphine-dependent rhesus monkeys. A similar severity of withdrawal was induced by 14-hr deprivation or precipitated by naloxone, naltrexone, cyclazocine, Win 44,441 or MR 2266. Regardless of the procedure used to induce withdrawal, behavioral signs were completely suppressed by a cumulative dose of 17.5 mg/kg morphine. Thus, an equivalent level of withdrawal induced by reversible antagonists is as sensitive to subsequent morphine administration as is deprivation-induced abstinence. This is in accordance with the theory that vacancy of opiate receptors normally occupied by morphine is related to the level of abstinence observed. In contrast to Win 44,441, however, an equivalent level of withdrawal precipitated by buprenorphine required 175 mg/kg morphine for complete suppression. This is more informative than comparing duration of action; when given as 24-h pretreatments, a high dose of Win 44,441 (10 mg/kg) was only slightly less effective than buprenorphine (3.2 mg/kg) in antagonizing morphine-induced stupor in normal monkeys. Comparison of the ability of morphine to suppress precipitated withdrawal provides evidence of the relative reversibility of antagonists in vivo and demonstrates the extraordinarily stable nature by which buprenorphine acts at opiate receptors.

INTRODUCTION

Narcotic antagonists have been extremely important historically in terms of the identification of opiate receptors, investigations of the homeostatic role of endogenous opioids, generally as tools in all areas of opiate research, and of course, clinically. Interest in antagonists has been further heightened by the introduction of long-lasting narcotic antagonists in opiate-addiction therapy [1], and the synthesis of opiate receptor alkylators such as beta-chlornaltrexamine [2]. This laboratory has been investigating various properties of opiate antagonists using behavioral preparations. In the present study, the morphine-dependent monkey was used to investigate the reversibility of novel opiate antagonists.

METHODS

Subjects Group housed monkeys trained to receive injections were tested weekly. Their overt behavior was monitored continuously throughout the test sessions by two experienced observers familiar with the individual animals. General muscle relaxation and stupor grades were given according to the descriptions in Table 1. Monkeys were made dependent by administering morphine sulfate (3 mg/kg, s.c.) every 6 h for at least 3 months. Higher withdrawal severity grades were given with an increase in the number and severity of withdrawal signs [3]. Suppression of withdrawal was tested starting 14 h after the last maintenance dose of morphine (deprivation-induced withdrawal), 0.5 h after naloxone or 1 h after buprenorphine, cyclazocine, MR 2266 ((-)-5,9-diethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan), naltrexone and Win 44,441 ((2α,6α,11S)-(-)-1-cyclopentyl-5-(1,2,3,4,5,6-hexahydro-8-hydroxy-3,6,11-trimethyl-2,6,-methano-3-benzozocin-11-yl)-3-pentanone methanesulfonate) (precipitated withdrawal). Morphine was given cumulatively every 30 min such that the dose increased by ½ or ¼-log units.

TABLE 1

Scales by which monkeys were graded for general muscle relaxation (according to the position animals take while sitting still) and stupor (according to the animals' response to external stimuli).

Grade	Muscle Relaxation
0	No observable muscle relaxation
1	Slight facial relaxation, jaw slackening, shoulder droop
2	Pronounced facial relaxation, jaw slackening, shoulder droop
3	Monkey must brace himself to sit up
4	Monkey cannot sit
Grade	**Stupor**
NOS	No observable stupor
+	Monkey appears to stare into space
A	Monkey is inattentive to ordinary movements of other monkeys
A+	Monkey is inattentive to ordinary movements of observers
B	Must gain attention of monkey by loud noises
B+	Monkey responds only to opening of cage latch
C	Monkey responds only to loud noises by his ear
C+	Monkey responds only to touch

RESULTS

Normal Monkeys The duration of action of selected opiate antagonists were examined in drug-naive monkeys. Morphine (1-100 mg/kg) caused a dose-related increase in the number of monkeys showing higher levels of stupor and muscle relaxation. The effects of cumulative morphine were antagonized by 1 h pretreatment with naltrexone (1 mg/kg) or Win 44,441 (1 mg/kg). Morphine-induced stupor was also antagonized by 24-h pretreatment with Win 44,441 (10 mg/kg) or buprenorphine (3.2 mg/kg) as shown in Fig. 1; morphine-induced muscle relaxation was similarly antagonized. Twenty-four h pretreatment with naltrexone (10 mg/kg) did not antagonize the observable effects of cumulative morphine.

Dependent Monkeys An equivalent level of withdrawal (severity scores of 3.5-4.5) was observed during 14-h deprivation and after the administration of naltrexone (0.01 mg/kg), naloxone (0.03 mg/kg), cyclazocine (0.03 mg/kg), Win

44,441 (0.03 mg/kg) and MR 2266 (0.056 mg/kg) (Fig. 2). Withdrawal was completely suppressed by a cumulative dose of 17.5 mg/kg morphine. However, the same level of withdrawal precipitated by buprenorphine (0.3 mg/kg) required 175 mg/kg morphine for complete suppression.

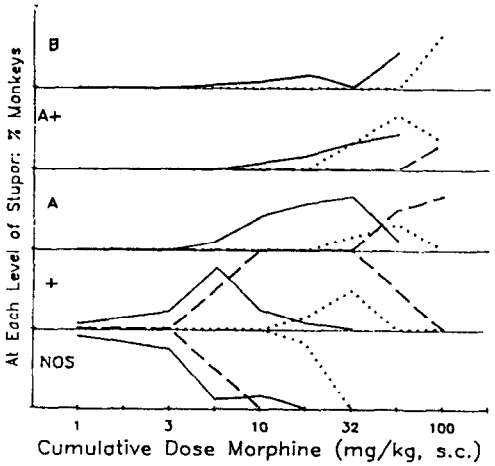

Figure 1: The effect of morphine given every 30 min to normal rhesus monkeys (n=6) on stupor in the absence of antagonist (solid lines), and 24 h after pretreatment with Win 44,441 (10 mg/kg; dashed lines) or buprenorphine (3.2 mg/kg; dotted lines). Each horizontal segment represents the percent of monkeys (from 0 to 100%) showing that particular level of effect indicated, as defined in Table 1.

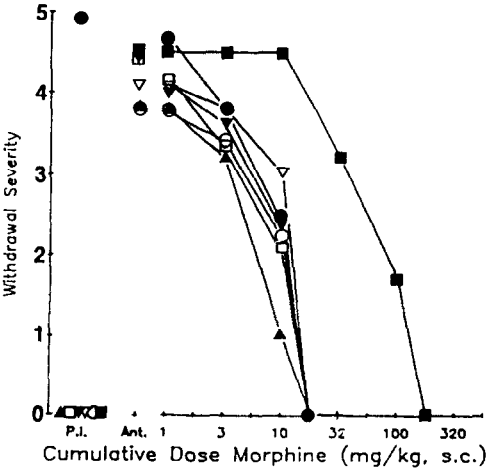

Figure 2: Morphine suppression of 14-h deprivation-induced abstinence (●) and withdrawal precipitated by naloxone (▲), naltrexone (□), MR 2266 (▼), cyclazocine (▽), Win 44,441 (○) and buprenorphine (■) in morphine-dependent rhesus monkeys (mean of 6). P.I.= pre-injection; Ant. = antagonist administration.

DISCUSSION

The dose of antagonist given to dependent monkeys can be adjusted such that the resulting withdrawal is of equivalent severity to that produced by a period of abstinence from morphine. Furthermore, there are no observable differences in the behavior of monkeys undergoing precipitated and deprivation-induced withdrawal. Given that the severity of effect is equivalent, deprivation-induced and precipitated abstinence is suppressed by equal amounts of morphine. This suggests that equivalent withdrawal is produced by the equivalent mechanisms, whether due to deprivation or antagonist administration, and extends the notion [4] that opiate receptor occupancy (i.e., the fraction of receptors no longer occupied by morphine) is related to the level of withdrawal observed.

Buprenorphine is a very lipophilic agonist-antagonist which dissociates extremely slowly from opiate receptors. Hambrook and Rance [5] suggested that this property accounts for the long duration of action of this compound and its relative resistance to antagonism. Whereas Win 44,441 and buprenorphine both have long durations of action in reversing morphine, ten times more morphine

was required to reverse the established antagonist effect of buprenorphine than Win 44,441 (above). Similarly, Cowan et al. [6] found that significantly higher doses of diprenorphine were required to reverse, rather than prevent, agonist effects of buprenorphine in the rat. These studies demonstrate the extraordinary stability of the buprenorphine-receptor complex. The fact that buprenorphine-induced withdrawal could be reversed by subsequent morphine administration, however, indicates that buprenorphine is not absolutely insurmountable in this system, whereas beta-funaltrexamine-induced withdrawal, for example, cannot be reversed by morphine [7]. Suppression of withdrawal is a useful and simple procedure for the in vivo estimation of the reversibility of antagonists.

In summary, the results indicate that (a) abstinence severity is related to the vacancy of opiate receptors normally occupied by morphine and (b) the suppression of withdrawal by morphine provides information as to the relative reversibility of opiate antagonists.

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