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A Single-dose Suppression Test in Morphine-dependent Mice[1] (37987)

A. E. TAKEMORI, ALAN J. STESIN, AND F. CANKAT TULUNAY[2]

Department of Pharmacology, University of Minnesota, Health Sciences Center Medical School, Minneapolis, Minnesota 55455

During the evaluation of a number of potential nonequilibrium analgesic receptor inactivating compounds (1), the need arose for a simple screening test for the prediction of dependence liability. Although the single-dose abstinence suppression technique has been used successfully in monkeys for a number of years in Seever's laboratory (2), this technique has not been exploited extensively in smaller species. Also the single-dose suppression method in monkeys did not appear to predict the dependence liability in man of some classes of compounds, notably the benzomorphans (3).

In the morphine-dependent mouse, a peculiar abstinence sign is an apparently uncontrollable urge to jump (4). This withdrawal jumping behavior has been shown in Way's laboratory to be a sensitive indicator of the degree of dependence (5). The jumps have been quantitated by a simple quantal method (5) and by observation of number of jumps (6, 7). Using the all-or-none measure, Way *et al.* (5) had shown previously that a single dose of a number of narcotic analgesics including a benzomorphan drug, could suppress withdrawal jumping in morphine-dependent mice while other central nervous system depressant drugs could not. In the present paper, we describe a more sensitive single-dose suppression test based on a quantitative method for assessing withdrawal jumping in morphine-dependent mice.

Methods and Materials. Male Simonsen mice weighing 20–25 g were used in all experiments. The animals were treated subcutaneously with 50 mg/kg morphine sulfate, t.i.d. for two days and 100 mg/kg, t.i.d. on the third day prior to subcutaneous implantation of morphine pellets. Each pellet contained 75 mg of morphine base and the entire composition was described earlier (8). The pretreatment with injections of morphine prior to pellet implantation reduced the mortality rate and assured us that at least 70% of the mice would exhibit the withdrawal jumping behavior. Seventy-two hours after pellet implantation, the pellet was removed and the area was irrigated with saline solution to assure complete removal of the pellet debris. Six hours after the removal of the pellets the mice were color coded with felt tip markers and placed in a 45 × 45 cm Plexiglas cylinder five at a time, and the number of animals jumping as well as the number of jumps for each animal was recorded for 15 min. A response was considered a jump when all four feet left the floor at the same time. In confirmation with the observations of Way *et al.* (5), maximum percentage of animals jumped between 6–8 hr after pellet removal. Also the maximum number of jumps per animal occurred during this time interval. The animals were then injected subcutaneously with one of the test drugs for the single-dose suppression test. In each set of experiments, three test drugs were evaluated and 8–10 mice were injected for each test drug. The test drugs as well as saline solution were all coded and the test was performed blindly by the experimenter. One hour after the administration of the test drug, the animals were

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[2] Visiting Postdoctoral Fellow from the Department of Pharmacology, University of Ankara, Faculty of Medicine, Ankara, Turkey.

again observed for their jumping behavior for 15 min as described above.

Fifteen to seventeen hours after the observation of withdrawal jumping, animals in each test group were subdivided into groups of 4–6 animals and the ED50 of naloxone was determined by the up and down method of Dixon (9). The end point was a simple quantal method of whether the mouse jumped or not within 15 min after each intraperitoneal dose of naloxone. The experimenter also tested these animals blindly, i.e., he did not know which drug the mice had previously received the day before.

The nonparametric method of Wilcoxon (10, 11) for paired differences was used to statistically analyze the number of jumps before and after the test drug. Chi square analysis was used where quantal data are presented.

Morphine sulfate and methadone hydrochloride were obtained from Mallinckrodt Chemical Works, levorphanol tartrate and dextrorphan tartrate from Hoffmann-La-Roche, Inc., GPA 1657 (1-1,2,3,4,5,6-hexahydro-3,11-dimethyl-6-phenyl-2,6-methano-3-benzazocin-8-ol·HCl) and GPA 1658 (d-isomer) from Geigy Chemical Corp., aminopyrine from K & K Laboratories, Inc., nalorphine hydrochloride and anileridine hydrochloride from Merck and Co., Inc. and naloxone hydrochloride from Endo Laboratories, Inc. Solutions of the drugs were made in saline solution such that 10 ml/kg were administered subcutaneously.

Results. A number of known narcotic, non-narcotic and narcotic antagonists were tested by the described method and the results are recorded in Table I. Using the all-or-none measure for withdrawal jumping, relatively high doses of all narcotic drugs significantly suppressed the incidence of jumping. However, when graded doses of morphine or methadone were tested, incidence of jumping was not significantly suppressed at doses below 10 mg/kg. On the other hand, when the number of jumps per animal was statistically analyzed, highly significant suppression of the number of jumps was observed even at a dose as low as 1 mg/kg of morphine. The administration of saline, aminopyrine or the antagonists, nalorphine and naloxone did not suppress the withdrawal jumping. Both antagonists appeared to slightly increase the mean number of jumps per animal. The experimenter was also able to show that the inactive optical isomer of a morphinan derivative, dextrorphan and a benzomorphan derivative, GPA 1658 were not capable of suppressing withdrawal jumping.

Additional information from the same experimental animals were obtained the next day by determining the ED50 of naloxone for precipitating jumping behavior. Inspection of Table I reveals that in some instances, the up and down method for small samples do not yield close, repeatable ED50 values. Nevertheless the mean ED50 gives a general indication of the degree of dependence in the animals of various groups. Generally, mice which previously received compounds that did not suppress withdrawal jumping exhibited ED50 values greater than one. This is seen in the cases where the animals had previously received saline, aminopyrine or the inactive isomers. Mice which previously received analgesics that suppressed withdrawal jumping exhibited ED50 values of 0.5 mg/kg or less indicating that the degree of dependence in these groups of animals was greater than the above groups. ED50 values of above 3 mg/kg were observed in those mice which had previously received narcotic antagonists indicating that the loss of dependence had been hastened and the degree of dependence in these animals were less than the saline-treated control animals.

Discussion. The single-dose suppression test appears to be a sensitive test for the prediction of narcotic dependence liability. The sensitivity is heightened by counting the number of withdrawal jumps as opposed to an all-or-none measure. This is clearly apparent when graded doses of the narcotics are used in the suppression test. For example, methadone at 5 mg/kg does not significantly suppress the incidence of withdrawal jumping which is in agreement with Way *et al.* (5). However, when the number of jumps are statistically analyzed by nonparametric methods, this dose of metha-

TABLE I. Single-Dose Suppression Test in Morphine-Dependent Mice.

Test drug and dose	No. mice jumped / No. mice tested Before	After	Chi square analysis P	Mean No. of jumps per mouse[a] Before	After	Wilcoxon analysis for paired difference P	ED50 of Naloxone mg/kg ± S.E.[b]	N[c]
Saline (control)	13/18	13/18	0.99	73.5	75.2	>0.1	1.25 ± 0.54	4
Morphine 10 mg/kg	16/18	3/18	<0.001	70.3	4.7	<0.01	0.26 ± 0.07	4
Morphine 5 mg/kg	7/10	3/10	>0.05	79.6	7.6	≦0.02	0.28 (0.15, 0.40)	2
Morphine 2.5 mg/kg	7/10	4/10	>0.1	72.6	10.1	<0.01	0.52 (0.44, 0.60)	2
Morphine 1.0 mg/kg	9/10	6/10	>0.1	76.0	18.0	<0.01	0.46 (0.44, 0.47)	2
Anileridine 25 mg/kg	8/10	0/10	<0.01	53.1	0	≦0.01	0.32 (0.44, 0.20)	2
Methadone 10 mg/kg	7/ 9	0/ 9	<0.01	51.7	0	≦0.02	0.17 (0.23, 0.11)	2
Methadone 5 mg/kg	7/10	2/10	>0.05	64.2	5.1	<0.01	0.39 (0.46, 0.32)	2
Levorphanol 4 mg/kg	7/10	0/10	<0.01	46.5	0	≦0.02	0.28 (0.30, 0.26)	2
Dextrophan 4 mg/kg	7/10	7/10	>0.5	40.4	30.1	>0.1	1.34 (0.79, 1.90)	2
GPA 1657 2.5 mg/kg	22/30	6/30	<0.001	62.8	4.5	<0.01	0.28 ± 0.07	6
GPA 1658 2.5 mg/kg	22/30	17/30	>0.1	49.8	40.4	>0.1	0.87 ± 0.28	6
Nalorphine 10 mg/kg	7/10	7/10	>0.5	105.3	111.2	>0.1	3.07 (4.75, 1.39)	2
Naloxone 1 mg/kg	8/10	9/10	0.99	59.9	71.3	>0.1	3.04 (2.30, 3.78)	2
Aminopyrine 100 mg/kg	8/10	7/10	0.99	68.5	70.6	>0.1	1.25 (1.50, 0.99)	2

[a] Non-jumping mice were not included.

[b] In cases where less than 4 groups of animals were used to determine the ED50, the average and the individual values in parenthesis are given.

[c] Number of groups used to determine the ED50.

done clearly suppresses the number of jumps. This dose also visibly decreases the severity of other signs of abstinence notably the increased locomotor activity and defecation.

The validity of the present test is strengthened by the fact that no false negatives resulted, i.e., compounds known not to support narcotic dependence does not show up to be a positive suppressor of morphine abstinence. This test also differentiated between the benzomorphan optical isomers, GPA 1657 and 1658. In single-dose suppression studies in morphine-dependent monkeys, GPA 1658 suppressed the abstinence syndrome while the analgesically active isomer, GPA 1657, did not (3). GPA 1658 was also found to substitute for morphine and initiate responding for self-administration in rats, while GPA 1657 did neither (12). It appeared therefore that GPA 1657 was a potent analgesic with little or no potential dependence capacity while GPA 1658 possessed dependence liability with little or no analgesic activity. Unfortunately in man, GPA 1657 was judged to be typically morphine-like and to have significant abuse potential (13). Results of the present study show that perhaps the mouse-model should be considered for more reliable predictions on the dependence potential properties of the benzomorphan-type analgesics.

The familiar Straub tail phenomenon and analgesic activity produced in mice by narcotic drugs have been offered as a rapid screening test for the prediction of dependence liability (14). One feature of the dependence of the morphine-type is that when animals become dependent on one narcotic drug they are invariably cross-dependent on all the other narcotic analgesics. Thus, the main advantage the single-dose suppression test has over the above ones is that it measures directly the suppression of abstinence due to morphine dependency.

Finally, the use of the mouse as the test subject has obvious economical advantages over usage of larger species. There are no maintenance problems, morphine dependency is produced in just a few days and the test is simple enough to be performed by relatively untrained personnel. Additional information as to the degree of dependency in the same experimental animals can be obtained with just a little additional effort of determining ED50 of naloxone.

Summary. A simple single-dose suppression test based on a quantitative method for assessing withdrawal jumping in morphine-dependent mice has been established for the prediction of dependence liability. When various narcotic, non-narcotic and narcotic antagonist agents were coded and tested blindly, the experimenter was able to identify all the drugs correctly.

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