A SCORING SYSTEM FOR ABSTINENCE SYNDROME IN MORPHINE DEPENDENT MICE AND APPLICATION TO EVALUATE MORPHINE TYPE DEPENDENCE LIABILITY OF DRUGS

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Abstract—Mice were made physically dependent after 10 days morphinization and subjected to natural withdrawal. The spontaneously developing signs in behavior were classified depending on type and frequency and utilizing same a scoring system was developed. The scoring system permitted an approximate appraisal of the rise and fall of abstinence state up to 10 hr after the start of withdrawal. When the single suppression technique was applied, it thus became possible to generally estimate the dependence liability of a given drug and at the same time, to determine whether or not a drug could be substituted for an opiate thereby predicting the liability of morphine-type dependence.

Recently, the mouse has been qualified as a suitable animal model for testing physical dependence liability of morphine-type drugs. Among a number of abstinence signs firstly described by Maggiolo and Huidobro (1--2) with this animal species, jumping syndrome was demonstrated by Way et al. (3) to be a reliable indicator in quantifying the extent of physical dependence and, so far, the utility seems to have been endorsed by widespread use of the syndrome in many laboratories (4--12).

As reported in a previous paper (12), there is certain evidence, however, suggesting that the syndrome may not be the most sensitive indicator of physical dependence. When withdrawal was induced by naloxone, the syndrome could serve as a reliable indicator, even after a single dose of morphine, however, when the animal was subjected to a natural withdrawal, especially after short-term morphinization, development of the syndrome was rare, while other abnormal behavior prevailed, in the majority of the animals. In addition, there is the fact that naloxone induced jumping is difficult to be masked completely by supplying the opiate in the dose range computed from the usual agonist to antagonist potency ratio (11--12). On the other hand, the jumping syndrome is not specific to the opiate dependent mouse as it has also been observed in barbital dependent groups (13). Furthermore, as described below, the syndrome could be masked by employing relatively high doses of other central nervous system depressants. For these reasons, the abstinence syndrome should be evaluated as a whole pattern consisting of multiple types of behavior.
It was attempted herein to characterize and evaluate individual abnormal behavior ensuing from natural withdrawal after ten days morphinization and secondly, applying the single dose suppression technique, to develop a method which would indicate whether or not a given drug would provoke morphine-type dependence.

**MATERIALS AND METHODS**

Mice (* dd* strain, male, weighing 19 to 21 g at start of the experiment) were morphinized twice daily (9 a.m. and 6 p.m. around the clock) with daily increasing doses (10, 20, 40, 60, 80 and 100 mg/kg) of morphine hydrochloride for 6 days and with a maintenance dose (100 mg/kg) for the following 3 days. On the morning of the 10th day, five animals each were removed at random to form a group. Around 9 a.m., the animal was given the final maintenance dose of morphine hydrochloride and natural withdrawal began.

**Table 1.** Scoring system for withdrawal signs recorded on a group of mice.

| Appearance* rate | Jumping | Score for |
|------------------|---------|-----------|
|                  |         | Falling, biting or backward locomotion |
| 1                | 2       | 1         |
| 2                | 4       | 2         |
| 3                | 6       | 3         |
| 4                | 8       | 4         |
| 5                | 10      | 5         |

| Appearance* rate | Mean frequency*** |
|------------------|------------------|
|                  | 0-4 5-9 10-14 >15 |
| 1                | 0 1 2 3 -2 -1 0 1 |
| 2                | 1 2 3 4 -1 0 1 2 |
| 3                | 2 3 4 5 0 1 2 3 |
| 4                | 3 4 5 6 1 2 3 4 |
| 5                | 4 5 6 7 2 3 4 5 |

| Mean frequency*** | Score for washing, grooming or wet dog shake |
|-------------------|---------------------------------------------|
| 0                 | -1                                          |
| 1-4               | 0                                           |
| 5-9               | 1                                           |
| >10               | 2                                           |

* No. of the animal(s) which exhibited the sign.

** The score for a sign in the table is given depending on both appearance rate and mean frequency.

*** Mean frequency was calculated by dividing the total frequency recorded in a group of mice with 5.
The withdrawal signs recorded were classified according to the abnormality and frequency and a graded score was given for each (Table 1). The highest score was given to the sign which appeared only in the morphinized mice (Table 1a) and the medium grade to the sign which was not specific to the morphinized group but which significantly increased in frequency (Table 1b). The lowest score was given to a few types of behavior which slightly increased in frequency on withdrawal (Table 1c). The obtained score was totalled by a group for a given period of monitoring (20 min) and expressed as the sum of scores.

The last scheduled dose of morphine given to the morphinized group induced typical behavior expected from the known actions of the alkaloid, i.e. Straub tail, hypermotility, etc., but these effects subsided within 3 hr, after which the score started to rise, indicating the onset of the abstinence state. Thus, monitoring of the behavior was begun 3 hr after the last dose of morphine had been given. The intensity of the abstinence state reached a plateau 5 to 6 hr after the morphine administration. In order to test the suppressive effect of a drug, it was given 4.75 hr after the final morphine administration and, to the morphinized control group, saline was given. Throughout the experiment, the drug was given s.c. and the dose is expressed in terms of the salt.

RESULTS

About 3 hr after the final dose, the animals began to exhibit withdrawal signs and the sum of scores increased slowly with time until a peak was reached about 5 to 6 hr after the morphine dose (Fig. 1). Later the score gradually decreased and approached that of control.

Re-administration of morphine completely suppressed any type of behavioral signs within 15 min and the score fell to the control level. Depending on the dose of morphine

![Fig. 1. Time course of withdrawal scores after the final scheduled dose of morphine and effects of re-administered morphine. At the time indicated by the arrow, the morphinized mice were treated with saline (○—○) or morphine hydrochloride (20 mg/kg: ×—× and 50 mg/kg: ●—●). The control group were on saline (▽—▽).](image-url)
FIG. 2. Time course of withdrawal scores after the final scheduled dose of morphine and effects of methadone. At the time indicated by the arrow, the morphinized mice were treated with saline (○—○) or methadone hydrochloride (10 mg/kg: ✗—✗ and 20 mg/kg: ●—●).

FIG. 3. Time course of withdrawal scores after the final scheduled dose of morphine and effects of pethidine. At the time indicated by the arrow, the morphinized mice were treated with saline (○—○) or pethidine hydrochloride (20 mg/kg: ✗—✗ and 50 mg/kg: ●—●).

FIG. 4. Time course of withdrawal scores after the final scheduled dose of morphine and effects of phenobarbital. At the time indicated by the arrow, the morphinized mice were treated with saline (○—○) or sodium phenobarbital (20 mg/kg: ✗—✗, 50 mg/kg: ●—● and 100 mg/kg: ▽—▽).
re-administered, the extent and duration of suppression varied. Withdrawal signs eventually re-appeared and the score usually drew a second and delayed peak.

Similar patterns of suppression and re-appearance of the abstinence signs were recorded by administration of methadone or pethidine hydrochloride (Figs. 2-3). Soon after administration, pethidine transiently accentuated intensity of withdrawal signs but later suppressed them as did the other narcotics.

The suppressive effect was not limited to morphine and its surrogates. For example, barbiturates, if given in high doses, were found to completely suppress the signs. Fig. 4 shows the effect observed with phenobarbital, however, this effect was different in nature as compared to those of narcotics, as the abstinence syndrome never re-appeared even after the sedative effect had almost worn off.

**DISCUSSION**

As reported previously (12), short-term morphinization of mice failed to build up so strong a physical dependence as to be detectable in the form of the jumping syndrome on natural withdrawal. Though naloxone precipitated the syndrome in those animals, the syndrome was not completely suppressed by a re-administration of morphine in a dose range computed to be reasonable. Mechanism for the failure of an opiate to protect mice against naloxone-precipitated withdrawal was discussed by Cheney et al. (11), and it was concluded that this failure could not be explained in terms of a simple competition between the agonist and the antagonist.

Thus, inadequacy of the naloxone induced syndrome as the indicator in testing dependence liability of the drugs led to development of a morphinization method which stands on the following criteria: the method can induce a high grade of physical dependence which can be easily detected without precipitating abstinence signs by naloxone and secondly, can be carried out using relatively mild and unlaborious treatments. When the mice were morphinized according to the schedule used herein and the behavior monitored after every morning dose, it was found that the animals began to develop various detectable abstinence signs after 3 days treatment. The intensity of the signs tended to rapidly increase during the following 3 days but a relatively stable and constant level of abstinence state as being measured in terms of scores was reached while on the maintenance dose which lasted for another 3 days. In addition, that built up dependence waned gradually and, without another administration of morphine, withdrawal signs were observed to develop on the 11th day (unpublished data).

One of the difficulties encountered in assessing this potency of the abstinence syndrome was what type of behavior should be selected as being typical. Careful observations of animal behavior allowed selection and classification into three categories as shown in Table 1. Fig. 5 shows the time course of individual abnormal behavior recorded for 10 hr.

Huidobro and Maggiolo (2) have previously developed a simple scoring system for abstinence syndrome of this animal species but, as described by the authors themselves,
their system did not allow for subjective evaluation of intensity of abstinence state. For this reason, the method of scoring individual behavior has to be carefully selected. In our system, the highest score was given to the jumping syndrome, which is regarded as the most intensive sign, as in withdrawal after short-term morphinization, the appearance rate of the syndrome was near zero but increased with the length of morphinization time (3, 12). In addition, an attempt was made to compensate for the loss in score caused by the masking effect of the jumping syndrome on other signs. As seen in Fig. 5, other types of behavior disappeared while the animal was jumping. It is highly probable that, as the intensity of abstinence reaches a crucial point, concentration may occur to produce a precipitate, namely jumping.

In contrast to the signs included in Table 1b and 1c for which the score was calculated based on, principally, how many times a given sign appeared in a group of mice, the score for those in Table 1a was given depending on the number of the animal(s) which exhibited the sign. This is to exclude the possibility that the obtained score may reflect individual differences to an unreasonable extent. Indeed, it was usually observed that jumping was completely absent in some mice while others continued to jump until only an exhausted state prevented continuation. In the same way and probably based on a similar consideration, quantification of the syndrome has been made by other workers (3, 9). On the other hand, the signs listed in Table 1b and 1c were not specific to morphine dependent mice and then scored in a different way.

A typical time course of withdrawal scores recorded according to our system is outlined in Fig. 1. In some cases, however, the second peak appeared about 9 hr after the morphine dose, as shown in Fig. 5. A plausible explanation for the phenomenon is that, during the long lasting observation period, the animal became exhausted but later resumed
the activity or it may reflect a rhythm conditioned by repeated experiences of morphine treatment.

The scoring system which was developed here is not necessarily free from the charge of being an arbitrary one and it is not easy to prove that the obtained results follow exactly the actual time course of withdrawal. However, when withdrawal was reversed by a re-administration of 100 mg/kg of morphine, the suppressive effect was observed to persist for about 3 hr as seen from Fig. 1 and the duration was approx. the same as that of the agonistic effect of the previous dose given in the morning. It is considered, therefore, that the scoring system does follow, with close proximity, the time course of withdrawal.

When pethidine was similarly tested, the score was observed to rise transiently to a slight extent but later to fall to a lower level. The results seem to be in good agreement with those reported by Huidobro and Maggiolo (14) for the narcotic.

In our methods the behavior of the animal was observed for 10 hr, therefore not only the suppressive effect but also the substitutive effect of a given drug could be analyzed. As shown in Fig. 4, abstinence signs could be suppressed by other drugs, for example, by phenobarbital if given in a high dose. The nature of the effect was, however, judged to be nonspecific, namely not substitutive, as was reflected in the non-appearance of a delayed peak in score. In contrast, when the narcotics were tested, the action was purely substitutive and withdrawal signs resumed activity after the agonistic effect of the drugs had almost subsided.

Thus, the method described herein allows characterization of the type of suppressive action of the drug under study and at the same time to generally estimate the liability of morphine-type dependence, for example, by measuring the area enclosed between the curves of scores recorded by saline and drug treated groups.

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