SIGNIFICANCE OF WITHDRAWAL JUMPING RESPONSE IN PREDICTING PHYSICAL DEPENDENCE IN MICE*

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Accepted December 15, 1972

Abstract—The significance of the naloxone-induced jumping response in predicting the physical dependence capacity of morphine-like analgesics was investigated in mice treated with morphine, morphine-6-glucuronide and pentazocine. The jumping response was induced by naloxone in mice chronically treated with morphine and morphine-6-glucuronide, but its development is not necessarily related to the number or frequency of drug injections, and it was also observed even after a single dose of the drugs. The jumping was not precipitated in mice chronically treated with pentazocine. The naloxone-induced jumping response in mice treated with morphine was not masked by morphine, but was markedly suppressed by chlordiazepoxide, diazepam, methamphetamine, delta-9-tetrahydrocannabinol and diphenylhydantoin. It is concluded that the naloxone-induced jumping response is not a specific abstinence phenomenon in mice treated with morphine-like analgesics, although it may be used for a first screening test to estimate a physical dependence capacity.

Way et al. (1) suggested that the intensity of physical dependence in morphine-tolerant mice could be quantified by determining the percentage of animals which jumped off a platform after the injection of a morphine antagonist, naloxone. Saelens et al. (2) proposed that this procedure could be used as a screening test for estimating the physical dependence capacity of unknown compounds.

With respect to the significance of this jumping response in predicting the physical dependence capacity of morphine-like drugs, the present investigation was undertaken in order to clarify the relation of a frequency as well as an interval of morphine injections to naloxone-induced jumping response, and the influence of various psychopharmacological agents.

MATERIALS AND METHODS

Male dd strain mice, weighing 20 to 24 g, were used. The experiments were performed at room temp. 23 ± 1°C with a 65% relative humidity.

The jumping response was measured by modifying the procedure described by Way et al. (1).

* Results of this investigation were presented before the 23rd (September, 1970) and 24th (October, 1971) Southwest Regional Meetings of Japanese Pharmacological Society.

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et al. (3). Mice were challenged with naloxone hydrochloride at a dose of 4 mg/kg s.c. 6 hr after the last injection of morphine or other analgesics. For measuring the jumping response, 5 animals were simultaneously placed on a round platform 30 cm in diameter and 35 cm in height. The number of animals which jumped off the platform was calculated within 30 min after naloxone injections.

Analgesics used in this experiment included morphine hydrochloride, morphine-6-glucuronide (M-6-G) (4), which was synthesized chemically (5, 6), and pentazocine which were all injected s.c.

Psychopharmacological agents, the effects of which were tested on the jumping response, included chlorpromazine hydrochloride, perphenazine hydrochloride, reserpine, diazepam, chlordiazepoxide, imipramine hydrochloride, natural delta-9-tetrahydrocanabinol, diphenylhydantoin, sodium pentobarbital, atropine sulphate, mephenesin and pentetrazol. All drugs were dissolved in saline or appropriate solvents and were injected i.p. in a fixed volume of 0.1 ml/10 g.

RESULTS
1. Relation of the number and interval of injections of analgesics to naloxone-induced jumping response

Groups of 20 mice each were used for each dose of the drugs in these experiments. Morphine at a doses of 40 and 80 mg/kg, M-6-G at a dose of 5 mg/kg, and pentazocine at a dose of 75 mg/kg were injected s.c. repeatedly at different intervals of 8, 12 and 24 hr. The incidences of naloxone-induced jumping response in each group are illustrated in Fig. 1. In groups on morphine and M-6-G at 8 hr intervals, occurrence of the jumping response was found to be related to the number of injections: i.e. the incidence of jump-

![Graph showing jumping response in mice after repeated doses of morphine, M-6-G and pentazocine administered at various intervals (N=20).](image-url)
ing increased according to the number of injections. In groups on these drugs at intervals of 12 and 24 hr, however, the incidence of jumping response did not increase in parallel with the number of injections.

Jumping response was absent in animals on pentazocine for any interval (Fig. 1).

2. **Naloxone-induced jumping response after a single dose of analgesics**

The jumping response was measured by challenging with naloxone, 1, 3 and 6 hr respectively after a single injection of morphine and M-6-G at various doses, in groups of 20 mice each. Results are summarized in Table 1. The jumping response was induced by naloxone even in mice on a single dose of the drug, although the incidence was lower than in animals on repeated injections. In addition, naloxone-induced symptoms other than jumping were characterized by urination, while defecation and hyperactivity were not significant which differed from those on repeated administration.

**Table 1. Jumping response in mice after a single injection of morphine and M-6-G.**

| Drug | Dose (mg/kg, s.c.) | Incidence of jumping |
|------|------------------|---------------------|
|      |                  | 1  | 3  | 6 hr |
| Morphine | 40 | 0/20 | 2/20 | 0/20 |
|         | 80 | 2/20 | 4/20 | 2/20 |
|         | 160 | —  | —  | 1/20 |
| M-6-G | 5   | 2/20 | 8/20 | 4/20 |
|         | 10 | —  | —  | 8/20 |
|         | 20 | —  | —  | 4/20 |

— : not tested

**Table 2. Effects of morphine on the jumping response in morphine-treated mice.**

| Treatment                      | Incidence of jumping | Mean number of jumps per mouse |
|--------------------------------|----------------------|-------------------------------|
| Control (naloxone)             | 17/20                | 10.8                          |
| Morphine (60 min prior to naloxone) | 10/10               | 12.8                          |
| Morphine (simultaneously with naloxone) | 14/20               | 10.7                          |
| Morphine (15 min after naloxone)  | 9/10                | 15.1                          |

3. **The effect of morphine on naloxone-induced jumping response**

In mice administered 14 injections of morphine 40 mg/kg s.c. at 8 hr intervals, morphine 40 mg/kg was injected s.c. 60 min prior to, simultaneously with and 15 min after naloxone administration respectively. The jumping response was measured during a period of 30 min after naloxone injection. Results are shown in Table 2.

The jumping response was not masked by morphine in any group.
In mice administered 14 injections of morphine 40 mg/kg s.c. at 8 hr intervals, various psychopharmacological agents were injected i.p. 30 min prior to the jumping test. Effects of these drugs on the jumping response are summarized in Table 3.

Forty-five out of the 62 control animals (72.6%) exhibited withdrawal jumping after 14 injections of morphine. Minor tranquilizers such as chlordiazepoxide and diazepam markedly suppressed the jumping response, while on the contrary, major tranquilizers such as chlorpromazine, perphenazine and reserpine failed to block the jumping response even at higher doses, which caused definite sedation and catalepsy. Amitriptyline, an antidepressant, showed a dose-related effect in suppressing the withdrawal jumping, but the effect of imipramine was insignificant. Methamphetamine completely blocked the jumping response at doses of 5 and 10 mg/kg, but had no effect at a dose of 2 mg/kg.

Table 3. Effects of psychopharmacological agents on the jumping response in morphine-treated mice.

| Drug                      | Dose (mg/kg, i.p.) | Incidence of jumping (%) |
|----------------------------|--------------------|--------------------------|
| Saline                     |                    | 45/62 (72.6)             |
| Chlorpromazine             | 10                 | 7/10 (70.0)              |
|                            | 20                 | 10/10 (100.0)            |
| Perphenazine               | 0.5                | 6/10 (60.0)              |
|                            | 1                  | 4/10 (40.0)              |
|                            | 2                  | 7/10 (70.0)              |
| Reserpine                  | 10                 | 7/10 (70.0)              |
| Chlordiazepoxide           | 1                  | 3/10 (30.0)              |
|                            | 2                  | 1/10 (10.0)              |
|                            | 5                  | 1/12 (8.3)               |
| Diazepam                   | 2                  | 5/10 (50.0)              |
|                            | 5                  | 1/10 (10.0)              |
|                            | 10                 | 1/10 (10.0)              |
| Imipramine                 | 10                 | 10/19 (55.6)             |
|                            | 25                 | 5/10 (50.0)              |
| Amitriptyline              | 5                  | 6/10 (60.0)              |
|                            | 10                 | 4/10 (40.0)              |
|                            | 20                 | 2/10 (20.0)              |
| Methamphetamine            | 2                  | 7/10 (70.0)              |
|                            | 5                  | 0/10 (0.0)               |
|                            | 10                 | 0/10 (0.0)               |
| Natural delta-9-tetrahydrocannabinol | 5 | 0/10 (0.0) |
|                            | 10                 | 0/10 (0.0)               |
| Diphenylhydantoin          | 20                 | 1/10 (10.0)              |
|                            | 50                 | 3/10 (30.0)              |
| Pentobarbital              | 20                 | 7/10 (70.0)              |
| Atropine                   | 5                  | 10/10 (100.0)            |
| Mephenesin                 | 50                 | 5/7 (71.0)               |
| Pentetrazol                | 10                 | 6/10 (60.0)              |
jumping response was also completely blocked by delta-9-tetrahydrocannabinol. Diphenylhydantoin suppressed the jumping response, though the effect was not dose-dependent. Pentobarbital, atropine, mephenesin and pentetrazol were without effect on the jumping response.

**DISCUSSION**

The present investigation confirmed the development of the naloxone-induced jumping response to be related to the number of morphine injections to mice on repeated doses of morphine 40 mg/kg at 8 hr intervals, as described by Way et al. (1). In mice on morphine at intervals of either 12 or 24 hr, however, jumping response was not related to the number of injections, and little difference was found in incidence of the jumping response between doses of 40 and 80 mg/kg. Since the jumping response was observed in the M-6-G group but not in the pentazocine group, it appears conclusive, that M-6-G does have a physical dependence liability while pentazocine does not, according to the suggestion of Saelens et al. (2).

However, the jumping response was found to be induced by naloxone even after a single dose of morphine and M-6-G, though the incidence was not so high as compared with that after a repeated administration. The question would therefore arise as to whether or not physical dependence on morphine could develop even after a single dose.

In an attempt to linealize the specificity of the naloxone-induced jumping response as an abstinence phenomenon, the effect on this response of various drugs including morphine was investigated.

The naloxone-induced jumping response was not masked by morphine administration in our experiment. Way et al. (3), on the contrary, reported that 5 mg/kg of morphine suppressed the withdrawal jumping response which appeared after removal of the morphine pellet in morphine-implanted mice. This could be explained by the difference between natural withdrawal and naloxone-induced withdrawal syndromes. Kaneto and Nakanoishi (7) also reported that the same jumping response induced by naloxone in morphine-infused mice could be completely masked by subsequent injection of morphine at doses of 20 to 100 mg/kg. At present, we are not prepared to offer an explanation as to why morphine did not suppress the jumping response. It is conceivable that naloxone antagonized morphine even to the point of subsiding the withdrawal syndrome.

Among various psychopharmacological agents, benzodiazepines, methamphetamine, delta-9-tetrahydrocannabinol and diphenylhydantoin were found to markedly suppress the naloxone-induced jumping response in mice after repeated administration of morphine. It is obvious that these drugs do not have a morphine-like dependence liability and cannot be substituted for morphine in morphine-dependants. Saelens et al. (2) reported that jumping could not be precipitated by morphine antagonists in mice chronically treated with chlorpromazine, chlordiazepoxide, imipramine and meprobamate. If this jumping response was a withdrawal phenomenon specific to narcotic analgesics, it could never be suppressed by these drugs.
From these experimental results, it is concluded that the naloxone-induced jumping response is not a specific abstinence syndrome in mice treated with morphine-like analgesics, although it may be used for a first screening test to estimate the physical dependence capacity of unknown compounds.

Acknowledgement: Naloxone was kindly provided by Central Research Laboratories, Sankyo Co., Ltd., Tokyo.

REFERENCES

1) WAY, E.L., LOH, H.H. AND SHEN, F.: Science 162, 1290 (1968)
2) SAELENS, J.K., GRANT, F.R. AND SAWYER, W.K.: Arch. int. Pharmacodyn. Thér. 190, 213 (1971)
3) WAY, E.L., LOH, H.H. AND SHEN, F.: J. Pharmacol. exp. Ther. 167, 1 (1969)
4) SHIMOMURA, K., KAMATA, O., UEKI, S., IDA, S., OGURI, K., YOSHIMURA, H. AND TSUKAMOTO, H.: Tohoku J. exp. Med. 105, 45 (1971)
5) YOSHIMURA, H., OGURI, K. AND TSUKAMOTO, H.: Chem. Pharm. Bull. 16, 2114 (1968)
6) OGURI, K., YOSHIMURA, H. AND TSUKAMOTO, H.: Chem. Pharm. Bull. 18, 309 (1970)
7) KANETO, H. AND NAKANISHI, H.: Japan. J. Pharmacol. 21, 411 (1971)