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A SIMPLE QUANTITATIVE METHOD FOR THE
EVALUATION OF PHYSICAL DEPENDENCE
LIABILITY OF MORPHINE IN MICE

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Previously, we reported that a continuous infusion of morphine hydrochloride into mice either subcutaneously or intravenously could establish "acute tolerance" to the analgesic effect of this alkaloid within a few hours, confirming the observation by Cox et al. (1), and also obtained some evidences suggesting the similarity of the tolerance produced in this way to that routinely acquired by repeated administration (2).

On considering the well known intimate relationship of tolerance with physical dependence, therefore, it would be the natural extension of our research to examine if the mice which developed distinct tolerance to morphine after a short-term infusion, had indeed become physically dependent on this alkaloid. In the same animal species implanted with morphine pellet, Way et al. (3) have recently succeeded in quantifying the intensity of developed physical dependence by estimating the dose of naloxone to precipitate withdrawal jumping syndrome. In the experiment reported here, thus, we adopted the jumping syndrome and the loss of body weight as the index of abstinence syndrome aiming at the evaluation of the intensity of physical dependence after continuous infusion of morphine in mice.

Adult male mice (dd-strain) weighing 20 ± 0.5 g were used. The animal was fixed in a plastic box specially designed for this experiment and the solution of morphine hydrochloride (10 mg/ml) in saline was infused subcutaneously or intravenously at the rate of 35 mg/kg/hour using a continuous infusion apparatus (Natsume KN-204) which was modified so as to treat ten animals at one time. The details of the experimental condition applied for the present study have been reported previously (2). The mice which received saline instead of morphine solution served as control. After infusion for 1, 3, 5, 7 or 10 hours, the mouse was discharged from the box, weighed and transferred onto a circular platform (30 cm in diameter and 45 cm in height). The changes in body weight at intervals of 30 minutes and the behaviour were recorded for the next three hours. Ten mg/kg

TABLE 1. Jumping test after abrupt withdrawal by 10 mg/kg of naloxone in continuously morphine infused mice.

Infusion* (hr)	Route	No. of animals	No. of jumped	Time after naloxone
1	s.c.	5	0	—
3	"	5	1	5—25
5	"	5	2	7—30
7	"	5	3	14—30
10	"	5	4	5—35
1	i.v.	5	1	5—35
3	"	5	0	—
5	"	5	1	10—15
7	"	5	4	5—15
10	"	5	2	2—24

* 10 mg/ml of morphine-HCl solution in saline was infused at the rate of 0.07 ml/hr (35 mg/kg/hr).

of naloxone hydrochloride solution in saline was given at 30 minutes after the discharge from the infusion apparatus.

During the first 30 minutes on the platform, no striking difference in behaviour was observed between saline- and morphine-infused groups, except the latter group continued to exhibit Straub's tail reaction uniformly which appeared soon after the start of infusion and lasted until the treatment of naloxone. The injection of naloxone to morphine-infused group caused intensive urination within one to two minutes and subsequently induced abnormal behaviours such as reported by Maggiolo and Huidobro (4). In contrast, control group did not show any specific reaction to this treatment. The most striking abnormal behaviour in the former group was the uncontrollable urge to jump as described by Maggiolo and Huidobro (4) and later confirmed by Way et al. (3). As shown in Table 1, the number of mice driven to jump increased in proportion to the infusion time in subcutaneously infused groups. In intravenously infused groups, extension of infusion time did not necessarily increase the frequency of jumping. Aside from jumping, however, some mice of this group ran out involuntarily from the edge of the platform, partly due to the unsuppressible hypermotility. All these abstinence syndromes which appeared following naloxone treatment in morphine-infused mice, could be completely masked by subsequent injection of morphine (20 to 100 mg/kg). On the other hand, an abrupt loss in body weight after withdrawal, mainly due to marked urination, was commonly observed in morphine-infused mice but to the similar extent (0.65–1.26 g/mouse) regardless of the duration of infusion. Therefore, this syndrome would not directly indicate the intensity of physical dependence though it could be rather a sign of physical dependence as we reported earlier (5).

These results obtained here seem to show that a short-term infusion of morphine could confer not only tolerance to but also physical dependence on this alkaloid to mice, and also to confirm the conclusion of Way et al. that the jumping syndrome can be used as a reliable index to quantify the degree of morphine dependence in this animal.

As a consequence of this study, the combined use of short-term infusion method and jumping test has allowed us to evaluate the dependence liability of other narcotic analgesic drugs in a simple manner. The details of those results will be given elsewhere.

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EFFECTS OF FENTANYL UPON THE SPINAL INTER- NEURONS ACTIVATED BY A δ AFFERENT FIBERS OF THE CUTANEOUS NERVE OF THE CAT

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In our previous papers (1, 2) it was shown that narcotic analgesics have common characteristics of selectively depressing or blocking the late phase of the inhibition of spinal extensor monosynaptic reflex evoked by the stimulation of either cutaneous or high threshold muscle afferent fibers. It was also demonstrated that the depressed phase of the cutaneous inhibition after narcotic analgesics was produced from A δ afferent fibers (1). Since these facts suggested that some spinal interneurons activated by such small fibers might be depressed by narcotic analgesics, it was attempted to find such neurons in the spinal cord of unaesthetized low spinal cats. All surgical operations were performed under ether anesthesia. The laminectomy was carried out from L₅-L₇ and the spinal cord was transected at T₅ under local anesthesia with procaine. In a few experiments, the spinal cord was also exposed from T₆-T₇ and a pair of Ag-ball electrodes were placed on the dorsal part of the lateral funiculus of this part for antidromic stimulation. A collar-type electrode was implanted on the ipsilateral quadriceps nerve for stimulation. Two of these were also attached to the ipsilateral saphenous nerve, one for stimulation at proximal and another for recording at distal part. After the operation was completed, anesthesia was discontinued and animals were immobilized with gallamine triethiodide and were maintained under artificial respiration. A glass pipette microelectrode filled with 1M K-acetate solution saturated with methylblue (1-10 M Ω) was inserted into the lumbar spinal cord, and the neuronal activity was projected on the cathode-ray oscilloscope (Taktronix 565)