LETTER TO THE EDITOR

Morphine analgesia, tolerance and dependence in mice from different strains and vendors

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In several of our papers concerned with the effect of various manipulations on the development of tolerance to and physical dependence on morphine in the mouse, a point of contention between us and the referees has been the differences in absolute values we have reported for the acute and chronic responses to morphine. In our rebuttal, we have pointed out that the strain differences in sensitivity to morphine can be considerable and that the validity of our findings and conclusions rests on assessments of relative changes between the control and test groups, on the same strain of animals obtained at the same time from a single vendor. The present communication provides evidence to this effect and we describe herein comparisons on the action of morphine on lethality, analgesia and tolerance and dependence development on two strains of mice from three vendors.

Four groups of male mice: ICR mice from Simonsen (Gilroy, California, source I) and Horton Labs (Oakland, California, source II), Swiss-Webster mice from Simonsen (source III) and Sasco (Omaha, Nebraska, source IV) 25–30 g were used. Mice were received on the same day and were maintained on standard laboratory chow and tap water and housed in rooms lighted artificially for 12 h of the day for one week before experimentation. Five criteria were used to assess strain differences: (1) determination of acute lethality of morphine (LD50); (2) cumulative mortality after morphine pellet implantation; (3) assessment of morphine analgesia (AD50) by the tail-flick reaction time to a thermal stimulus; (4) degree of tolerance development after 3 days of subcutaneous implantation of a 75 mg morphine pellet by the relative increase in the morphine AD50; and (5) assessment of physical dependence by determining either the incidence of spontaneous jumping 6 h after pellet removal (abrupt withdrawal) or by estimating the dose of naloxone (ED50) to induce the response (precipitated withdrawal). For complete descriptions of the procedures used see Way, Lon & Shen (1969).

There were some differences in the acute toxicity of morphine in the four groups of mice studied, but they were not striking. The order of significant differences is as follows: (source III > source I = source IV > source II). In Fig. 1, the cumulative mortalities after morphine pellet implantation for the different groups of mice are shown. Considerable variations between the same strains from different vendors were noted.

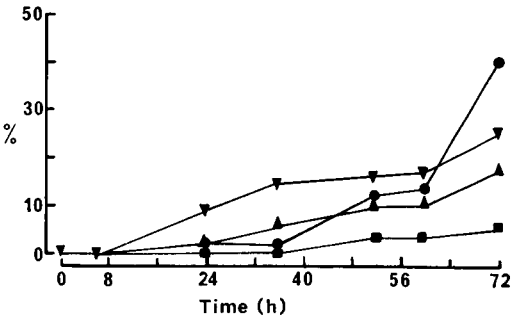

FIG. 1. Cumulative mortality (%) after morphine pellet implantation (h) in various strains of mice. The number of animals used in each group was between 60 to 77. ■—Source I. ▼—Source II. ●—Source III. ▲—Source IV.

Swiss-Webster mice from either source III or IV, were more sensitive to morphine in terms of antinociception as shown by their low morphine AD50 values. Table 1 shows that the morphine AD50 of naive ICR mice obtained from both sources (I, II) was about twice that of the Swiss-Webster mice obtained from both sources (III, IV).

After rendering the animals tolerant by morphine pellet implantation, however, the morphine AD50 was

Table 1. *Morphine AD50 in non-tolerant and tolerant mice of different strains.*

	Morphine AD50 mg kg^{-1}, s.c. (95% confidence limits) Non-tolerant	Tolerant	Tolerant/ Non-tolerant
Source I	6·0 (4·7–7·7)	41 (32·8–51·3)	6·9
Source II	7·3 (5·5–9·7)	52 (40·3–67·1)	7·1
Source III	3·3 (2·5–4·4)	54 (42·9–68·0)	16·4
Source IV	3·0 (2·3–4·0)	41 (33·3–50·8)	13·3

A quantal response to at least three doses of morphine was used to determine the median analgesic dose (AD50) and 95% confidence limits by the method of Litchfield & Wilcoxon (1949).

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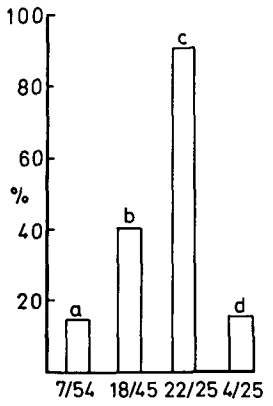

FIG. 2. Incidence of spontaneous jumping (%) 6 h after removal of morphine pellet in various strains of mice. a—Source II. b—Source I. c—Source III. d—Source IV.

not significantly different among the four groups. As a consequence, the degree of tolerance development to morphine in Swiss-Webster mice after 3 days of pellet implantation was about twice that of ICR mice. After mice became physically dependent, withdrawal jumping was induced by removing the morphine pellet. As seen in Fig. 2, the findings were remarkably varied with respect to strain, or even the same strain from different vendors. Marked differences in naloxone ED50's were also noted in mice from different sources. As shown in Table 2, the naloxone ED50 in ICR mice from source I 6 h after the removal of the morphine pellet, was only about 1/9th that of the animals obtained from source II. The naloxone ED50 of Swiss-Webster mice from source III could not be obtained since almost 90% of the dependent mice exhibited spontaneous withdrawal jumping.

We have used mice purchased from varying sources during the past seven years owing to the vendors not having an adequate supply to meet our demands. Since 1969 we have reported naloxone ED50's ranging from 0·04 to 1·6 mg kg^{-1}, subcutaneously. Although this may seem inconsistent, the findings on any one strain

Table 2. *Naloxone ED50 in different strains of morphine-dependent mice.*

	Naloxone ED50, mg kg^{-1}, s.c. (95% confidence limit)*
Source I	0·04 (0·03–0·05)
Source II	0·37 (0·28–0·48)
Source IV	0·25 (0·20–0·31)

* A quantal response to at least three doses of naloxone was used to determine the median effective dose (ED50) and 95% confidence limits by the method of Litchfield & Wilcoxon (1949).

of mice from the same vendor were fairly constant, e.g. if the studies were made with source II ICR mice, the naloxone ED50 remained between 0·4 and 1·0 mg kg^{-1}, subcutaneously, after development of dependence, while the naloxone ED50 of the source I ICR mice, obtained with the same techniques, was consistently about 1/10th that of the source II ICR animals.

The present studies demonstrate that different strains of mice obtained from the same supplier or the same strain of mice purchased from different vendors exhibited varying sensitivities to morphine in toxicity, analgesia, and the development of tolerance and dependence. Among the four groups of animals tested, Swiss-Webster mice from source III turned out to be most susceptible and sensitive to morphine; however, there were no other discernible trends within the data. It appears that the differences in morphine responses in the mouse might be due to two possible causes. When breeding stocks from different companies are allowed to inbreed without fresh stock from identical strains from other sources, strain divergence can result from mutations and introduce variations into the separated strain. Different environments at the farm (e.g., diet, hormones or antibiotics, if any) might also cause alterations in identical strains from differing sources.

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