Acute Morphine Tolerance in New Born and Young Rats*

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Abstract. The development of tolerance after two consecutive doses of morphine administered at 4 h intervals was studied in 16, 25 and 38 day-old Sprague-Dawley rats. Tolerance was measured by the diminution of the rats' response to morphine on a hot plate (56°C). The ages selected correspond to clear stages of the brain barrier permeability to morphine. A loss of sensitivity to morphine was observed according to age. Acute tolerance developed at all ages tested, except when higher doses were employed in 25 and 38 day-old rats. When acute tolerance had developed it persisted for a long time. It is concluded that the development of acute tolerance to morphine analgesia does not depend on the stage of development of the rat.

Key words: Morphine Tolerance — Sensitivity to Morphine — Morphine in Newborn — Acute Tolerance — Morphine and Development.

Introduction

Recent evidence has shown that tolerance to morphine analgesia is produced after a single dose or within hours of an infusion of morphine; this rapid decrease of morphine effect is termed acute tolerance, which is long-lasting and the development of which is blocked by protein synthesis and nucleic acids inhibitors (Cochin and Kornetsky, 1964; Lotti, Lomax, and George, 1966; Smith, Karmin, and Gavitt, 1967; Cox, Ginsburg, and Osman, 1968).

Inasmuch as morphine has a central site of action, it was thought of interest to see whether morphine tolerance is dependent on a stage of the animals' growth or on its nervous system development. Cochin (1970) has reviewed the hypotheses that had been proposed to explain morphine tolerance. The most acceptable among them postulate an alteration of morphine metabolism, feed-back control of neurotransmitter concentration, enzyme expansion, and an immunological process. All of these theories state that some specific protein is required for the development of tolerance. As in the newborn animal most functions depending

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on protein synthesis are under development, it was decided to investigate whether acute tolerance bears some relation to age. For this purpose, rats at different stages of development were tested for acute tolerance.

**Methods**

Sprague-Dawley rats from 1 to 38-days old of both sexes were employed. Each experimental group was formed by litters of 10 animals from the same mother. They were fed by their mothers until weaned, and the food and tap water were allowed ad libitum. Temperature was maintained at 24°C. At 1, 16, 25, and 38-days of age, morphine was injected to study the development of acute tolerance to analgesia. For body weight see Tables 1, 2, and 3.

Analgesia was determined by Woolfe and McDonald's technique (1944). When rats were placed on a hot plate at 56°C they licked their forelimbs or jumped off the plate after some seconds. This lapse corresponds to the analgesia reaction time. The maximal time allowed on the hot plate was 30 sec in order to avoid burnings. Animals were trained on the hot plate seven days before starting the experiments. Their behaviour on the hot plate was erratic till they were aged 14 days; afterwards, responses to noxious stimuli were very regular. Analgesic reaction time was determined 1/2, 1, 2, 3, and 4 h after a dose of morphine. This value was then expressed as “index of analgesia” \( \frac{A_{\text{obs}}}{A_{\text{max}}} \), where \( A_{\text{obs}} = \text{ART}_{\text{obs}} - \text{ART}_m; \) and \( A_{\text{max}} = \text{ART}_{\text{max}} - \text{ART}_m; \) \( \text{ART}_{\text{obs}} \) is the mean analgesic reaction time after morphine; \( \text{ART}_m \), the analgesic reaction time before morphine and \( \text{ART}_{\text{max}} \), the maximal time allowed on the hot plate. Adapted from Cox et al., 1968). The total analgesic effect of morphine was finally quantified through the area of analgesia (\( \text{AA} \)) which was calculated plotting the index of analgesia versus time in hours, and expressed in square units. When the \( \text{AA} \) was statistically inferior to control it implied tolerance development; values superior to control signified decrease or abolition of tolerance.

The Students' \( t \) value was calculated in each experimental group: \( p \) values below 0.01 were considered statistically significant. For practical purposes, differences of 20% or more in \( \text{AA} \) determined this limit.

The development of acute morphine tolerance was studied as follows: an inducer dose of morphine (ID) was administered to each group of animals; 4 h later, when analgesia had completely waned, the same dose was repeated to detect whether the ID had produced tolerance. In some cases (Table 3) a third dose of morphine was injected 48 h after the ID.

Morphine hydrochloride, expressed as the base, was dissolved in distilled water and injected i. p. in doses ranging from 1 to 15 mg/kg (see Results). One-day old rats received 1 mg/kg of morphine s. c. The volume injected was never larger than 1 ml. Sex did not influence analgesia nor tolerance development. No changes in analgesia or in the animals' behaviour was observed when rats were injected with saline.

**Results**

Results were classified according to the different ages of rats. In each of the following experiments, paragraph A refers to animals that received morphine for the first time; paragraph B, to those previously treated with morphine.