Development and Loss of Tolerance to Morphine in the Rat

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Abstract. The development of a differential tolerance to morphine was investigated with respect to the mean effective dose, the threshold dose of tolerance, the degree of tolerance after a fixed dose, and the speed of tolerance loss. The mean effective doses, the threshold doses of tolerance, and the degree of tolerance differed considerably from effect to effect, whereas in all tests tolerance loss remained the same. The mean effective doses were not correlated to threshold doses of tolerance, degree of tolerance, or to the loss of tolerance, but a strong correlation exists between threshold doses of tolerance and degree of tolerance to all effects measured. Consequences of these results upon current theories of tolerance are discussed.

Key words: Morphine — Tolerance — Rat

The repeated administration of morphine to rodents produces tolerance (Kalant 1977; Fernandes et al. 1977a, b; Mucha et al. 1978, 1979). To emphasize that the degree of tolerance to various effects differs (Fernandes et al. 1977a, b), the term differential tolerance will be used. The phenomenon of differential tolerance is not yet understood. Most of the current hypotheses of tolerance postulate an adaptive physiological or biochemical process to the molecular effects of the drug when the drug is present (physiological theories’, Kalant 1978). In contrast, the hypothesis of functional tolerance (Kalant et al. 1971; Kalant 1978) postulates an adaptive process to a drug-induced functional disturbance. The latter is assumed not to be simply connected with the presence of the drug in the organism, because the particular effects do not cause the same intensity of functional disturbance or, thus, different degrees of tolerance. If this assumption is correct, a close relationship between the effective doses and the threshold doses of tolerance (TDT) to particular reactions should exist. The present study was undertaken to test this hypothesis. Furthermore, it was investigated whether differential tolerance is associated with the speed of tolerance loss, which could vary from effect to effect. It was also tested whether a correlation between tolerance-inducing doses and the degree of tolerance to various effects contributes to the phenomenon of differential tolerance.

A prerequisite for answering this question is the quantitative determination of tolerance. Kalant et al. (1971) have shown that tolerance in rodents can be quantified only by parallel shifts of log dose-response curves induced by chronic treatment with a drug. In an earlier paper this procedure proved to be suitable for the determination of differential tolerance to morphine provided that the degree of tolerance was low or moderate, i.e., up to delta log 1.4, which means that a dose 25-times higher is necessary after chronic treatment to produce the same effect as in naive animals (Fernandes et al. 1977b).

Materials and Methods

Male adult Wistar rats (Hagemann, Hannover, FRG), weighing 200—250 g, were used. They were housed in groups of six to eight animals per cage at an ambient temperature of 22°—23°C and had free access to food and water. To avoid the effects of repeated testing on tolerance development, all animals were used only once. Morphine HCl (Merck, Darmstadt, FRG) was injected SC twice daily (9 AM and 5 PM) for up to 20 days. All experiments on test days were carried out at 9—11 AM, i.e., 3—4 h after starting the light period (6 AM—7 PM). The doses given refer to morphine HCl.

To minimize the influence of the pharmacokinetics of morphine during chronic treatment, peak effects were determined whenever possible.

Lethality. Rats were kept in groups of five in plastic cages (26 x 42 x 15 cm). Incidence of death was checked 24 h after the test dose of morphine.

Catalepsy. At 60 and 90 min after the test dose of morphine the front paws of the rats were placed on a wooden bar at a height of 7 cm. Preliminary experiments showed a maximum cataleptic reaction at either of these times. Holding time in seconds was recorded by means of a stopwatch for up to 120 s. The higher value (60 or 90 min) was taken as the peak effect.

Hot Plate. The hot plate test (Eddy and Leimbach 1953) was adapted for rats. The plate temperature was 58°C, the latency until the rats licked their paws or jumped was registered by means of a stopwatch before, as well as 20 and 40 min after morphine administration. The difference in latency before and after (20 and 40 min) administration of morphine was determined; the higher value of both taken as the peak effect. Rats that did not lick paws or jump within 20 s were removed from the plate and a latency time of 20 s was recorded.

Acetic Acid Writhing. Acetic acid (0.3 ml, 10%) was injected IP 40 min after the morphine test dose. The number of
writhing movements was counted during min 5 – 20 after acid injection.

**Tail Flick.** The light of a lamp was focused on the tail of a rat at a distance of 2 cm from the tail root. The intensity was adjusted so that naive animals reacted by struggling or vocalization within 5 s. By means of a stopwatch, reaction time was registered before, as well as 20 and 40 min after the injection of morphine and the difference in latency before and after morphine administration was determined. The higher value (20 or 40 min) was taken as the peak effect.

**Body Temperature.** The Wistar rats used in our experiments reacted to morphine only with hyperthermia in contrast to most other strains described in the literature. The probe of an electric thermometer (Atmos) was inserted 6 cm into the rectum and body temperature was measured for 1 min at 60 or 90 min after morphine administration. The greatest increase from baseline in body temperature of the two measurements was taken as the peak effect of the drug.

**Tilted Plane.** The rats were placed on a flat wooden plane (40 x 20 cm) in horizontal position. Over 8 s, it was gradually moved into upright position, until the animals started to slide. Untreated animals started to slide at an angle of 73° ± 3°. The sliding angle was measured before, as well as 60 and 90 min after morphine. The greatest variation from the initial drug value was used as the response on the tilted plane.

**Suppression of Stress-Induced Defecation.** Rats were stressed by placing them into a round plastic cylinder (diameter 5.8 cm, 18 cm long). Of saline-treated rats, 82% (18 of 22) defecated within 10 min after confinement. At 40 min after morphine injection the percentage of animals defecating within 10 min was recorded.

**Determination of MED.** MED (mean effective dose) were determined by regression analysis from the linear part of complete dose-response curves described previously (Fernandes et al. 1977b). Half (50%) of the maximum response within the curve was defined as MED. In the case of all-or-none responses, ED50 were determined by probit analysis (Cavalli-Sforza 1969), but for convenience they were designated MED in this paper. For comparison with the minimum tolerance-producing doses, it would have been desirable to determine the minimum effective doses. However, an exact determination was made uncertain by the nonlinear parts of the dose-response curves. Since the sequence of the 10% effective doses is the same as that of MED of the various effects, with the exception of hyperthermia, we suggest that the order of MED reflects largely the sequence of the minimum effective doses.

Furthermore, an analysis of our data which defined the response in different tests in another way (e.g., for catalepsy, by defining a threshold dose instead of a continuous response) resulted only in minor variations of MED.

**Determination of Degree of Tolerance.** The acute and chronic (after 32 mg/kg morphine twice a day) dose-response curves (Fig. 1) were tested for parallelism, and the degree of the shift was determined by analysis of variance or, in the case of an all-or-none response, by probit analysis. Details are described in an earlier paper (Fernandes et al. 1977b). After the method of Kalant et al. (1971), the degree of shift of the log dose-response relationships was taken as a measure of the degree of tolerance. In this way, a shift (delta log) of 1.0 means that, in chronically treated rats, the dose of morphine has to be tenfold higher to obtain the same effect as in naive rats.

**Determination of TDT.** Different doses of morphine were administered twice a day for 10 days. The responses after test doses were always between the acute and chronic dose-response curves tested for parallelism. Assuming that the responses were all on dose-response curves parallel to the acute one, the shifts (delta log) were read directly from the graph (Fig. 1).

The relationship between the dose of morphine given chronically and the delta log shift was calculated for each effect by means of least-squares linear regression (Fig. 2). The dose of morphine at which the degree of tolerance reached the value of delta log 0.0 was defined as the TDT.

**Loss of Tolerance.** Loss of tolerance follows an exponential function. Therefore, the shift delta log is presented following a further logarithmic transformation in which the rate of loss of tolerance could be determined by least-squares regression from a log delta log-time diagram (Fig. 4). The rats were treated for 20 days twice per day with 32 mg/kg morphine and the degree of tolerance was determined on days 1, 5, and 10 after ending treatment.

**Statistical Analysis.** Kendall’s correlation coefficient (τ, Campbell 1971) was used for measuring the correlation between MED, degrees of tolerance, and TDT.

**Results**

**Relationship Between MED, Degree of Tolerance, and TDT.** Table 1 lists the mean acute effective doses, the degree of tolerance after administration of 32 mg/kg morphine administered twice per day for a period of 20 days, and TDT.

For acute effects MED vary between log 0.27 mg/kg and log 1.64 mg/kg (0.54 – 43.6 mg/kg). In different tests the same dose of morphine administered repeatedly also resulted in different degrees of tolerance, i.e., tolerance developed