Log Dose/Response Curve Flattening in Rats After Daily Injection of Opiates

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Abstract. Rats injected (IP) daily with 0, 20, and 200 mg/kg morphine-SO₄ for 25–49 days experienced log dose/response (LDR) curve flattening (decrease in slope and/or maximum response) for analgesia (tail immersion test) produced by etorphine-HCl injected IP or intracerebroventricularly (ICV), and for latency to maximum rectal temperature increase produced by IP etorphine. Rats treated similarly with 0, 50, and 500 µg/kg etorphine-HCl for 32 days exhibited LDR-curve flattening for analgesia produced by etorphine and morphine (IP). In addition, a profound body weight loss produced by high-dose morphine treatment (200 mg/kg) was found not to be involved in flattening, since similar body weight decreases produced by food restriction in 0 and 20 mg/kg rats did not have this effect. Flattening, however, may be due to a rapidly acquired and rapidly lost within-session (acute) tolerance. When flattening was not seen at short intervals after IP or ICV test etorphine doses, flattening was seen when rats were retested at longer test intervals. Forty-eight hours after cessation of chronic etorphine treatment, flattening of the etorphine analgesia LDR curve was lost, but parallel shift was unaffected. Similarly, 200 mg/kg morphine-treated rats lost morphine tolerance more rapidly than 20 mg/kg-treated rats during the first 12 days after the last treatment injection. Subsequently, however, levels of the analgesia and the amounts of tolerance loss were comparable in both chronically treated groups. The data support the notion that chronic tolerance reflects an enhancement or prolongation of acute tolerance.

Key words: Opiates — Analgesia — Body temperature — Cross tolerance — Acute tolerance — LDR curve — Flattening — Tolerance loss — Intracerebral injection

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Opiate tolerance is precisely defined as a parallel shift of the log dose/response curve to the right (Fernandes et al. 1977a, b). The parallel shift has been observed for various responses such as analgesia (Way et al. 1969; Fernandes et al. 1977a, b), latency to onset of hyperthermia (Gunne 1960), intestinal stimulation (Burks et al. 1974), contractions of electrically-stimulated ileum (Shoham and Weinstock 1974), and facilitation of avoidance behavior (Holtzman 1974). Moreover, parallel shifts have been observed with both morphine (Gunne 1960; Fernandes et al. 1977a) and pentazocine (Holtzman 1974).

Recent reports suggest that in addition to the parallel shift to the right, opiate tolerance may comprise an LDR-curve flattening. This is generally seen as a decreased slope of the LDR curve over low test dose ranges and a decrease in the maximum response obtainable when a number of high doses are employed (Mucha et al. 1979). Flattening occurred after morphine administration by pellet implantation (Theiss et al. 1975; Hui 1976) and by intraperitoneal (IP) injection daily (Mucha et al. 1978) or twice a day (Cox et al. 1975), in both rat (Cox et al. 1975; Theiss et al. 1975; Mucha et al. 1978) and mouse (Hui 1976). Moreover, flattening correlated well with the intensity of the naloxone-precipitated withdrawal syndrome (Mucha et al. 1979).

The main purpose of the present study was to determine whether flattening of the LDR curve is a general characteristic of opiate tolerance. This was done in five different ways.

(1) Previous demonstrations of flattening involved only morphine as the treatment drug. Therefore, treatment was carried out with etorphine, a morphine-like opiate agonist with pharmacokinetic properties markedly different from those of morphine (Herz 1973).

(2) Cross-tolerance between different opiates, as demonstrated with parallel shift (Seevers and Deneau 1963; Coper 1978), was tested in the phase of LDR-
curve flattening; etorphine cross-tolerance was studied in morphine-treated rats and morphine cross-tolerance in etorphine-treated rats.

(3) The test drug was given by a different route. In previous experiments demonstrating flattening, opiates were administered only systemically during testing. Administration of opiates into the brain produces analgesia (Mayer and Price, 1976), and it is generally agreed that most of the functional changes leading to tolerance to opiate effects are within the central nervous system (Seevers and Deneau 1963). Therefore, testing for flattening of analgesia LDR curves in morphine-treated rats was carried out with intracerebroventricularly (ICV) administered etorphine.

(4) The response generality of LDR-curve flattening was tested by using latency to maximum hyperthermia as a measure of opiate effect. Previously, flattening was found with the radiant heat tailflick test (Hui 1976), the tail immersion test (Mucha et al. 1978), paw pressure test (Cox et al. 1975), and shock-induced vocalizations (Theiss et al. 1975). However, these all involve responses to noxious stimuli. Flattening was also found in the immobility test (Mucha et al. 1978), which does not involve an aversive stimulus, but immobility may be a primary behavioral component of the morphine effect in many “analgesia” tests (Mucha et al. 1978). In contrast, latency to maximum hyperthermia measures on opiate effect of an altogether different type, and the measure is in terms of duration rather than magnitude of response. Despite this, it was shown to have a sigmoid LDR curve, which shifted to the right after treatment with morphine (Gunne 1960).

(5) Finally, the present study examined whether body-weight reduction produced by chronic opiate administration influenced the change in slope of the LDR curves. In the present experiments and in those published elsewhere (Mucha et al. 1978, 1979), there was a marked depression of normal weight gain with regimens of etorphine and morphine administration that caused LDR-curve flattening. Since LDR-curve flattening was found after administration of higher doses over more prolonged periods than those required to produce a parallel shift, flattening might have been a product of the severe physical insult caused by the treatment and unrelated to the cause of the parallel shift of the curve. Other data in the literature were consistent with this possibility. In mice, implantation of a single 75 mg morphine pellet caused weight loss (Way et al. 1969) and flattening of the LDR curve (Hui 1976). In rats, implantation of a single morphine pellet caused neither weight loss (Cicero and Meyer 1973) nor LDR curve flattening. Flattening was, however, produced by 21 pellets implanted over 9 days during daily surgery (Theiss et al. 1975). In addition, Cox et al. (1975) found flattening after injecting morphine twice each day, a regimen also producing weight losses (Martin et al. 1963). The role of weight loss in LDR-curve changes was therefore studied by measuring analgesia LDR curves following body weight decreases produced by food restriction in rats under morphine treatment regimens that do not normally cause LDR-curve flattening.

Additional information was collected regarding the nature of LDR-curve flattening. First, the time course of the etorphine effect produced by the test injection was measured for temperature change, and for tail-flick latency after both IP and ICV administration. Typically, demonstrations of LDR curve flattening have employed only single injection-test intervals. Comparisons among our own experiments have suggested that flattening is most readily observed with drug-test intervals of more than 1 h. Second, Cox et al. (1975) found an apparently rapid loss of morphine-produced flattening following seven days of withdrawal; however, no attempts were made to measure loss of the shift to the right over this same withdrawal period. Therefore, we studied the effect of withdrawal on both types of opiate-produced LDR curve.

Materials and Methods

Animals. Wistar rats, obtained from Canadian Breeding Laboratories (Constant, Quebec), were used at body weights of 325–475 g in the various experiments. Prior to the experiment they were housed singly with continuous access to food and water, in a temperature-controlled room with lights on from 0800–2000 h. Body temperature measurements were taken in a room with a temperature of 24°C. Male rats were used in all experiments except experiment 4 where the subjects were ovariectomized females. The ovariectomies had been carried out 3 months earlier for another study that was not carried out, and the rats were available at the time of the present experiments. Pilot studies indicated no critical differences between males and ovariectomized females with respect to the present findings.

The rats used in Experiment 3 were surgically implanted, under sodium pentobarbital (60 mg/kg), with unilateral ICV guide canulae, consisting of 13 mm of 22 gauge syringe tubing embedded in 8 mm of threaded plastic. The stereotaxic bregma coordinates were AP = 1.0, L 1.7, and V 4 mm (from dura), with the guide perpendicular to the surface of the skull. The guide was protected with a 30 gauge stainless steel obturator and a plastic cover that screwed onto the body of the guide. Shortly after the experiment, the animals were heavily sedated with pentobarbital and the cannula placements were verified by injecting 3 μl of methylene blue through the guide cannula, 5 min before decapitation. The data from an individual rat were included in the analysis if, upon removal and dissection of the brain, there was an even distribution of the dye through the ventricular system.

Drugs. Doses of morphine sulfate (British Drug Houses – BDH) and etorphine hydrochloride (Lederle) were expressed in terms of their salt. The solutions were made with saline and injected IP, 6 – 8 h into the light cycle. ICV administration was done with a 50 μl Hamilton syringe and a 30 gauge stainless-steel injector needle, cut to extend 0.5 mm beyond the guide. The injection volume was 10 μl, administered over a 5-s interval, and the needle was held in place for an additional 10 s. The IP injection involved 4 – 10 ml/kg of fluid.