Absence of Cross-Tolerance to Heroin in Morphine-Tolerant Mice

Abstract. *Mice implanted with morphine pellets demonstrated a 30-fold increase in tolerance to subcutaneously administered morphine but showed no cross-tolerance to subcutaneously administered heroin. When given morphine intracerebroventricularly, the mice showed no tolerance to morphine or cross-tolerance to heroin. These observations depended on the presence of the morphine pellet. If the pellets were removed prior to determinations of potency, the expected responses—tolerance to morphine and cross-tolerance to heroin—were obtained. The blood-brain barrier may be a prime site for the expression of morphine tolerance in mice.*

Long-term administration of narcotic analgesics characteristically leads to the development of tolerance and cross-tolerance to a variety of their pharmacologic actions (*1*). In general, such tolerance and cross-tolerance in animals is induced by a narcotic drug administered through systemic or central routes on a particular schedule (*2-5*). Alternatively, a pellet containing the tolerance-inducing agent may be implanted subcutaneously for a certain period and then removed before the test for tolerance or cross-tolerance is made. Removal of the pellet allows the animals to eliminate residual narcotic before an evaluation of the analgesic effects is made. We modified these tolerance-inducing procedures slightly, and our results have caused us to question previous interpretations of measurements of cross-tolerance. In our protocol, a morphine pellet implantation technique (*6*) was used to induce narcotic tolerance in mice (*7*), but we did not remove the pellets before measuring narcotic tolerance and cross-tolerance. This change in protocol was possible because the animals returned to their original sensitivity to a painful stimulus 72 hours after implantation of the morphine pellets (*8*).

By using this induction protocol and measuring analgesia by changes in the latency of tail flicking (*9, 10*), we observed a 30-fold increase in tolerance to morphine. Although Patrick *et al.* (*8*) observed a similar degree of tolerance in mice implanted with morphine pellets, the tolerance we measured was greater than that commonly reported by investigators who remove the pellet before measuring tolerance. Also, we were surprised to note that subcutaneous administration of etorphine to mice implanted with morphine pellets did not result in the expression of cross-tolerance to etorphine (*11*). We suspected that etorphine's greater potency [due to its high lipophilicity (*12*) and its high affinity for opiate receptors (*13*)] was partially responsible for this lack of observable cross-tolerance. To determine the effect of receptor affinity and drug lipophilicity, we investigated the effect of morphine pellet–induced tolerance on the analgesic potency of heroin. Heroin, the diacetyl analog of morphine, was selected because, like etorphine, it has a more lipophilic character than morphine (*1, 14*) and because, unlike etorphine, it must be metabolized to morphine in the central nervous system (CNS) in order to express its major analgesic activity (*15*).

In mice that were implanted with placebo pellets for 72 hours, the median effective dose (ED_{50}) for subcutaneous morphine was 4.5 mg/kg; in mice implanted with morphine pellets for an equal period, the ED_{50} was 127.0 mg/kg (Table 1). Conversely, the ED_{50} for subcutaneously administered heroin in control animals was the same as that for subcutaneously administered heroin in mice implanted with morphine pellets for 72 hours. Thus no cross-tolerance to heroin was produced by the morphine pellet as long as it remained in the animal. This absence of cross-tolerance to heroin is paradoxical, since heroin is thought to exert its analgesic effect by conversion to morphine in the brain. Perhaps a dispositional mechanism differentially excludes morphine from the brains of mice implanted with morphine pellets. This hypothetical mechanism is different from those suggested by other investigators (*16, 17*) because it appears to be specific for morphine relative to heroin and etorphine. This specificity and the unusually high tolerance expressed toward morphine may be linked to the nonremoval of the tolerance-inducing morphine pellet.

To further substantiate the differential dispositional effect of morphine pellet–induced tolerance to the analgesia produced by subcutaneously administered morphine and heroin, we injected both morphine and heroin intracerebroventricularly. Mice were anesthetized with halothane, and a small incision was made into the midline of the scalp to facilitate the location of skull features. Heroin or morphine, dissolved in 4 μl of 0.9 percent NaCl, was then injected through the skull into the lateral ventricle (*18*). The injection volume was kept at 4 μl to minimize the effects of transient injection pressure on the CNS and the amount of drug lost due to refluxing of the solution along the needle tract. The success rate for these injections, as determined by perfusions with India ink, was greater than 90 percent. The analgesic effect was measured 10 minutes after each injection (*19*).

When morphine was administered in this manner to control mice, the ED_{50} was 0.5 μg (Table 2). When morphine was given intracerebroventricularly to mice implanted with morphine pellets, the ED_{50} did not change. The ED_{50} for intracerebroventricularly administered heroin was the same for control mice and mice implanted with morphine pellets. Thus the latter did not express tolerance or cross-tolerance to intracerebroventricularly administered morphine or heroin while the morphine pellet remained in place. This result was similar to that reported by Way (*17*). After increasing the daily doses of morphine for 7 weeks, Way found that mice showed less tolerance to morphine when the route of administration was intracerebroventricular rather than intravenous. These observations support a mechanism of tolerance that is based on differential disposition, and suggest that the blood-brain barrier may be the prime site for the expression of tolerance to morphine in mice.

Since tolerance and cross-tolerance are usually measured after discontinuation of the drug, we measured the sensitivity of mice to morphine and heroin 3 hours after removing morphine pellets by the method described in (*20*). As before, the pellets had been in place for 72 hours. The ED_{50} for subcutaneously administered morphine decreased to 32.5 mg/kg, whereas the ED_{50} for subcutaneously given heroin rose to 3.1 mg/kg (Table 1). Thus after pellet removal the tolerance to morphine decreased and there was sudden cross-tolerance to heroin. A similar effect on the ED_{50} for intracerebroventricularly administered morphine and heroin was also seen upon removal of the morphine pellet (Table 2). Both ED_{50} values increased approximately threefold. Thus the lack of tolerance and cross-tolerance to intracerebroventricularly given morphine and heroin in mice implanted with morphine pellets rapidly changed to tolerance and cross-tolerance when the morphine pellets were removed.

Why were our results so different from those obtained by other investigators? One factor may have been our decision to test for tolerance and cross-tolerance without removing the morphine pellet.

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This was suggested by the tolerance data for animals whose morphine pellets were removed before tolerance evaluations were made. The rapid decrease in tolerance to subcutaneous injections of morphine was coupled to a rapid appearance of cross-tolerance to subcutaneous injections of heroin. This "withdrawal tolerance" to heroin appeared after both its subcutaneous and intracerebroventricular administration. The term "withdrawal tolerance" describes the animal's change in narcotic sensitivity after removal of the morphine pellet, when the morphine supplied by the pellet is rapidly leaving the brain (*8*). The animal's response to the decrease in morphine concentration in the brain is characterized by a variety of withdrawal signs. However, our interest is in the effect of the decreasing morphine concentrations on the ED_{50} of a subsequently administered narcotic. We suggest that a portion of the test analgesic is required to replace the morphine lost from the brain as a result of pellet removal. This replacement is required in addition to the morphine normally needed to produce analgesia, and may be responsible for the increase in the ED_{50} for heroin given subcutaneously after morphine pellet removal. Thus a significant component of the cross-tolerance phenomenon may be secondary to the effects of withdrawal from the narcotic drug used to induce tolerance.

When a narcotic drug is administered systemically on a scheduled basis to induce tolerance, animals undergo frequent and large fluctuations in the concentration of the narcotic agent in the brain (*21*). The tests for tolerance or cross-tolerance are generally conducted at the time of the next scheduled drug administration—a time when the concentration of the drug in the brain is probably decreasing rapidly. This loss parallels the rapid decline in morphine concentrations produced by morphine pellet removal. The universal observation of narcotic cross-tolerance in animals given narcotics systemically on a scheduled basis is not surprising.

The relation between morphine pellet removal and the appearance of a withdrawal tolerance to heroin may also explain the tolerance and cross-tolerance to narcotic analgesics seen after their scheduled administration by intracerebroventricular injection. Reports by Jacquet and Lajtha (*5*), Watanabe (*4*), and Eidelberg and Barstow (*3*) indicate that tolerance develops rapidly to both intracerebroventricularly and systemically administered narcotics. However, the induction of tolerance by intracerebroventricular means was always conducted on a schedule that may have produced withdrawal features prior to the tolerance tests. Thus the change in the ED_{50} values may have been due to the development of withdrawal tolerance.

Table 1. Median effective dose of subcutaneously administered morphine and heroin in mice with morphine pellet not removed or 3 hours after removal, showing absence of cross-tolerance and appearance of withdrawal tolerance. The 95 percent confidence interval for each value is given in parentheses.

Condition	ED_{50}*	
	Morphine (mg/kg)†	Heroin (mg/kg)‡
Control	4.5 (3.3 to 6.2)	0.60 (0.44 to 0.80)
Pellet not removed§	127.0 (77.3 to 208.0)‖	0.58 (0.38 to 0.90)
Pellet removed¶	32.5 (20.6 to 51.3)‖	3.2 (2.1 to 7.5)‖

*Each ED_{50} value was determined from a minimum of 40 animals (*10*). †Median effective dose was determined 20 minutes after subcutaneous administration. ‡Median effective dose was determined 15 minutes after subcutaneous administration. §Morphine-base pellet (75 mg) was implanted for 72 hours. ‖$P <$ .05, Student's *t*-test. ¶After being implanted for 72 hours, the pellet was removed 3 hours before administration of the narcotic agent.

An apparent inconsistency in the mechanism of withdrawal tolerance is the decrease in the ED_{50} for subcutaneously administered morphine as the morphine concentrations in the brain decrease after removal of the morphine pellet. This decrease in morphine's subcutaneous ED_{50} contradicts the increases in the ED_{50} for subcutaneously administered heroin and intracerebroventricularly administered heroin and morphine. We suspect that a withdrawal tolerance to morphine is expressed when the pellet is removed, but that the primary mechanism of tolerance acts to obscure its detection. Testing for analgesia before morphine pellet removal may be a better way of measuring narcotic tolerance than testing after morphine pellet removal. Since a considerable amount of morphine remains in the brain when the pellet is not removed, the return to normal sensitivity to pain and normal ED_{50} to intracerebroventricularly administered narcotics may be accurate indicators of tolerance. As a result, the concentration of morphine in the brain after the animal's return to a normal sensitivity to pain may be the best measure of narcotic tolerance.

The ED_{50} values for heroin and morphine given intracerebroventricularly to mice implanted with morphine pellets suggest that a 30-fold increase in tolerance to subcutaneously given morphine is not the result of an alteration in opiate receptor function. If a change in opiate receptors were responsible for the expression of morphine tolerance, then animals implanted with morphine pellets should show a similar increase in ED_{50} values for narcotics given intracerebroventricularly. Similarly, a change should be expected in the ED_{50} for subcutaneously given heroin. The absence of this increase in ED_{50} values supports studies in which no significant changes were found in opiate receptor binding affinity in morphine-tolerant animals in vitro (*22*) and in vivo (*23*).

The increase in the ED_{50} for subcutaneously administered morphine after the induction of morphine tolerance by pellet implantation may demonstrate a decrease in morphine's ability to reach the site of its activity in the CNS. The loss of accessibility appears to be somewhat specific for morphine in that the ED_{50} values for subcutaneously given etorphine and heroin were not altered, suggesting normal access to their CNS sites. It is conceivable that a variety of other narcotic and nonnarcotic compounds have an altered disposition in the CNS after the induction of morphine tolerance.

Table 2. Median effective dose of intracerebroventricularly administered heroin and morphine in mice with morphine pellets not removed or 3 hours after removal, showing absence of cross-tolerance and the appearance of withdrawal tolerance. The confidence interval, at a 95 percent level, is shown in parentheses.

Condition	ED_{50}*	
	Morphine (μg)	Heroin (μg)
Control	0.5 (0.4 to 0.8)	3.2 (2.0 to 5.0)
Pellet not removed†	0.4 (0.2 to 0.9)	3.5 (2.4 to 5.5)
Pellet removed‡	2.8 (1.2 to 6.4)§	9.8 (6.7 to 11.4)§

*Determined 10 minutes after intracerebroventricularly administered heroin or morphine in 4 μl of 0.9 percent NaCl. Each ED_{50} value was determined from a minimum of 40 animals. †Morphine-base pellet (75 mg) was implanted for 72 hours. ‡After being implanted for 72 hours, the pellet was removed 3 hours before administration of the narcotic agent. §$P <$ .05, Student's *t*-test.

In conclusion, a dispositional mechanism, possibly associated with changes in the permeability of the blood-brain barrier, may be responsible for the expression of morphine tolerance as represented by an alteration in the ED_{50} for subcutaneously given morphine. In addition, the observation of cross-tolerance to the highly lipophilic narcotic heroin after removal of a tolerance-inducing morphine pellet may be associated with the phenomenon of withdrawal tolerance. This tolerance, as characterized by an increase in the ED_{50} of systemically administered narcotics, should be distinguished from the central or neuronal tolerance that is demonstrated by normal sensitivity to a painful stimulus and normal ED_{50} for heroin and morphine administered intracerebroventricularly in the presence of high levels of morphine in the CNS.

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Formamidine Pesticides: Octopamine-Like Actions in a Firefly

Abstract. *The formamidine pesticide chlordimeform and its* N*-demethylated metabolites cause the light organ of the firefly* Photinus pyralis *L. to glow brightly. Monodemethyl chlordimeform is active at doses as low as 5 nanograms per insect when applied topically. This action is postsynaptic and probably involves membrane-bound receptors since cyproheptadine blocks the glows induced by both monodemethyl chlordimeform and octopamine, the putative neurotransmitter in the light organ. The pesticidal and pestistatic properties of the formamidines may result from actions on octopaminergic systems.*

The formamidines are a relatively new class of pest control agents effective against lepidoptera, certain other insects, and mites and ticks (*1*). Their effectiveness in plant and animal protection results, at least in part, from the induction of abnormal behavior in the pest rather than by direct lethality. Reduced feeding, dispersal from plants, erratic mating behavior, and detachment of ticks from their host are typical of these behavioral effects (*1-3*). Such actions have been termed pestistatic (*2*) rather than pesticidal. Several biochemical actions, including mitochondrial uncoupling, inhibition of monoamine oxidase, blockage of cholinergic neuromuscular transmission, and local anesthetic effects have been proposed to explain various facets of the toxicology of these agents (*2-5*). However, none of these can plausibly explain their striking effects on invertebrate behavior (*2-4*). We have found that the formamidine chlordimeform and some of its metabolites are potent effectors of the lighting response of the photocytes of the firefly *Photinus pyralis* L. This response is believed to be controlled by octopaminergic neurons (*6, 7*). In combination with other evidence, this result raises the possibility that interactions with octopaminergic

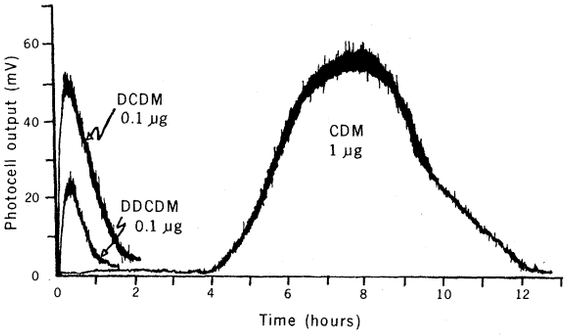

Fig. 1. Light output from adult fireflies treated topically with chlordimeform (*CDM*) or with two sequential *N*-demethylation products (*DCDM* and *DDCDM*). Acetone-treated controls gave no response.

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