

**AN INCREASED PRODUCTIONS OF MORPHINE-6-GLUCURONIDE, THE ACTIVE METABOLITE OF MORPHINE, IS CAUSED BY REPEATED HEROIN ADMINISTRATIONS IN THE RAT**

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Biotransformation of morphine mainly consists in the glucuronidation of either the phenolic (position 3) or the alcoholic (position 6) hydroxyl residue of the phenanthrenic molecule by UDP-glucuronosyltransferase (UGTP), an enzyme located in the lumen of the endoplasmic reticulum. The unexpected finding that M6G is more potent than the parent compound as an analgesic, whereas M3G is almost completely devoid of pharmacological effects, has prompted a reevaluation of the issue of morphine metabolism. In normal individuals, more M3G is formed than M6G (M3G/M6G ratio = 4-5). In contrast, we have recently found a reduced M3G/M6G ratio in heroin addicts relative to subjects acutely or chronically treated with morphine to reduce post-surgical pain. This phenomenon may depend on the chronic exposure to heroin or to some of the many pollutants that taint street heroin. For instance, cadmium has been found in street heroin at concentrations that inhibit M3G synthesis *in vitro*. The goal of the present study was to investigate the effects of chronic exposure to heroin and to cadmium chloride (CdCl<sub>2</sub>) on morphine glucuronidation in the rat.

Two groups of male SD rats received 10 daily i.p. injections of either morphine or heroin (10mg/Kg), respectively. On day 10, other two groups of rats, which had been previously injected daily with saline, received morphine or heroin (10mg/Kg, i.p.), respectively. Two hours after the last treatment, all animals were sacrificed by decapitation to collect blood for the quantification of morphine, M3G, and M6G. Liver, brain and kidneys were excised to obtain microsomes preparations. The activity of the microsomal UGTP was determined using morphine as substrate (0.1-4. mM). Concentrations of morphine and their metabolites were assessed using HPLC. In a second experiment, a similar procedure was applied to four groups of rats repeatedly injected with saline, heroin (10 mg/kg), CdCl<sub>2</sub> (10 mcg/kg), and heroin plus CdCl<sub>2</sub> respectively.

Repeated heroin increased M6G to detectable blood levels (whereas M6G was undetectable in all other groups) and reduced M3G levels relative to all the other treatment groups. Repeated heroin treatment was also associated with a significant reduction of  $V_{max}$ , but not of  $K_d$ , for the formation of M3G by liver microsomes. Most interestingly, a low capacity and a low affinity enzymatic activation of M6G synthesis was detected in the repeated heroin group only. No effects were observed on the enzymatic activity of brain and kidney microsomes. Cadmium, given alone or in combination with heroin, caused a significant reduction of M3G synthesis without activating M6G formation.

In conclusion, at the best of our knowledge, this is the first study in which chronic exposure to heroin has been evaluated as a factor influencing morphine glucuronidation. Its main result is that heroin caused a significant reduction of  $V_{max}$  of M3G formation by liver microsomes, and the activation of M6G formation. The daily administration of a dose of cadmium compatible with metal concentrations found in street heroin samples, reduced  $V_{max}$  of M3G formation by liver microsomes. However, unlike heroin, cadmium did not elicit M6G synthesis, suggesting a different mechanism of action.