Heroin Metabolism in the Rat[1] (37589)

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It is recognized that heroin produces more pronounced pharmacological effects and is more addictive than morphine (1). Heroin (3,6-diacetylmorphine) is deacetylated to 6-monoacetylmorphine (MAM) and then to morphine (2, 3). We have established a protocol for the administration of heroin and the study of its metabolic products in rats with the aid of radioactive tracers. This study was designed to attempt to elucidate the differences in rate of heroin deacetylation between three groups of animals: those who received heroin; those who received heroin and were withdrawn for 7 wk; and control animals. Emphasis is placed on very early points in time after injection of heroin.

Materials. Morphine (*N*-methyl-^{14}C) hydrochloride (sp act, 57 mCi/mmole) is available commercially. Morphine-^{3}H was prepared by New England Nuclear from cold morphine using tritium labeled trifluoroacetic acid. The specific activity of the crude material is 24.8 mCi/g. The compound is purified by column chromatography as described below. Since radioactive heroin is not available, it is synthesized starting with ^{3}H- or ^{14}C-morphine, according to the methods of Wright (4) and Small and Lutz (5). Cold heroin is synthesized by the same process starting with morphine HCl. The sample is dissolved in methanol and subjected to purification by column and thin layer chromatography (see Methods), followed by gas–liquid chromatography. Before experiments were performed, repeated checks of purity were determined by gas–liquid chromatography using a Varian 2100 gas chromatograph with columns of Dexsil 300, 15% on Anachrom Q 90–100 mesh (Analabs, North Haven, CT). The conditions are: temperature of column 250° programmed to rise 4°/min to 350°, injector 275°, detector 375; sensitivity 16 $\times$ 10^{-11}; flow rate of nitrogen 45 ml/min, air and hydrogen 300 and 30 ml/min, respectively.

The choice of Sprague-Dawley male rats is supported by data presented by Furner, Gram and Stitzel (6). The number of animals at each point ranged from 6 to 10.

Methods. Known amounts of ^{3}H-heroin were injected intraperitoneally into a male Sprague-Dawley rat. After varying intervals of time, a rat was decapitated, exsanguinated into a tube containing heparin, and the brain, kidneys and liver were removed and weight was determined. Known amounts of morphine-^{14}C were added to the tissues in saline to correct for losses due to manipulative procedures. The tissues were placed immediately in tubes of 1–2 ml of iced saline. Trichloroacetic acid was added to a final concentration of 10%. After homogenization, the suspension was adjusted to pH 4.5 with 5 *N* NaOH and 0.5 ml acetate buffer (pH 4.5). One gram of NaCl was added to each tube. Tissues were extracted three times with 1,2-ethylene dichloride:isobutanol (3:1) [2–3 $\times$ aqueous volume] (7). The organic extracts were pooled and dried *in vacuo,* dissolved in 1 ml 0.1 *N* HCl and chromatographed on a Celite 545 (Johns Manville, New York NY) column acidified with 0.1 *N* HCl. Aliquots were removed from column eluates for counting in a Nuclear Chicago Isocap 300 liquid

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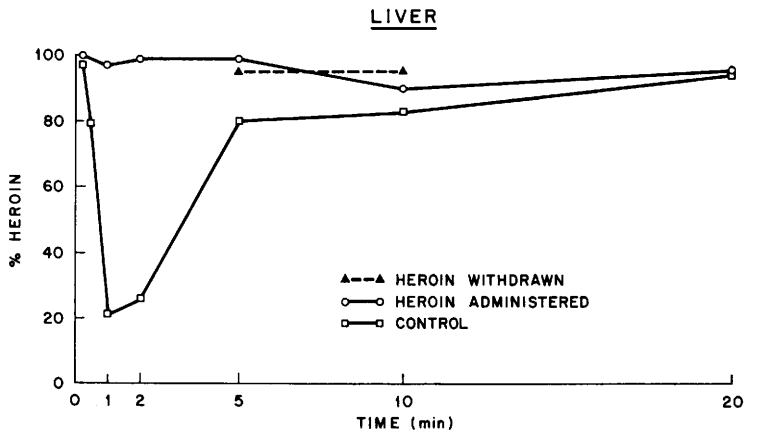

FIG. 1. Concentration of heroin present in *liver* as percentage of total radioactivity (^{3}H-heroin + ^{3}H-MAM + ^{3}H-morphine) after injection of ^{3}H-heroin. Comparison of control (□), heroin administered (○), and heroin withdrawn (▲).

scintillation system. The $^3H/^{14}C$ efficiencies are 48% and 70%, respectively, when counted to a 5% error (2000 cpm) in a double isotope program setting with external standard ratio and chemical quench correction curves.

The eluates containing morphine and MAM are dried under vacuum, dissolved in methanol, and applied to silica gel then layer chromatography plates. The solvent system used to develop the plates is methanol:*N*-butanol: benzene:H_2O, (60:15:10:15), (v/v) (7).

The radioactive peaks are located by a Varian Berthold radio scanner.

Experiment design. Heroin administered (HA). Young male Sprague–Dawley rats (100–150 g wt) received daily doses of cold heroin intraperitoneally starting with 0.2 mg/kg and rising 0.1 mg/day to a maintenance level of 0.7 mg/kg. This final dose is related to the average amount of heroin used daily by human addicts on a weight per weight basis.[2] The rats were sacrificed on Day 12, after challenge with ^{3}H-heroin (2 hr after receiving cold heroin 0.7 mg//kg).

Heroin withdrawn (HW). A group of rats were injected with heroin in the same manner as the HA group. At the end of the injection period, no heroin was administered for 7 wk before challenge with ^{3}H-heroin and sacrifice.

Control rats were sacrificed after injection with ^{3}H-heroin.

All rats received pellet food and tap water *ad libitum*. The cages were installed in a private room with light and darkness on a 12 hr cycle.

Results. The results are discussed as the percentage of ^{3}H-heroin present in relation to total tritium radioactivity (*i.e.*, ^{3}H-heroin + ^{3}H-MAM + ^{3}H-morphine).

All data are calculated as mean and standard error of the mean. Student's *t* test was used to calculate *p* values.

Figure 1 illustrates the obvious differences in heroin metabolism in the liver between control and HA rats. The controls show an immediate reduction in the presence of heroin. Within 5 min the heroin levels rise but never quite reach the same concentration as with HA rats. The livers of HA rats maintain high concentrations of heroin during the first 5 min postinjection. The difference between control and HA is significant at 1–2 min and 5 min with $p < 0.001$ and < 0.005, respectively. It should be noted that the control values are consistently lower than the HA at every point in time. The HW liver concentrations are parallel to the slope of the controls, although in the range of the HA levels.

In brain tissue, Fig. 2, the control values show heroin decreasing within 2 min. In the HA rats, brain levels are 97–99% over the

[2] Personal communication from Dr. Herbert Kleber, Chief, Drug Dependence Unit, Connecticut Mental Health Center, Yale University School of Medicine, New Haven, CT.

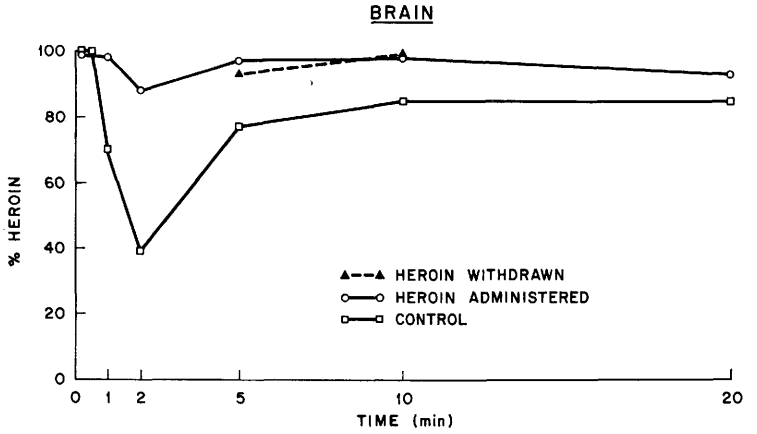

FIG. 2. Concentration of heroin present in *brain* as percentage of total radioactivity (^{3}H-heroin + ^{3}H-MAM + ^{3}H-morphine) after injection of ^{3}H-heroin. Comparison of control (□), heroin administered (○), and heroin withdrawn (▲).

first 10 min, except for a drop to 88% at 5 min.

The difference between control and HA is significant at 2 min ($p < 0.02$).

The kidneys of control rats (Fig. 3) show an immediate drop in the presence of heroin in 30 sec, slowly rising by 5 min. At 10 min, heroin is reduced, but rises at 20 min. In the HA rats, the unusual rise at 10 min is in inverse relationship to the drop in heroin concentration with control kidneys. Both HA and control were nearly identical at 5 min, with levels of 65 and 61%, respectively, and equal at 61% in 20 min.

The difference between HA and control kidney levels at 10 min was significant with $p < 0.005$. The significant differences at 1 and 2 min have $p < 0.001$ and < 0.02. The HW heroin levels for kidney parallel the downward slope of the controls while remaining 8–13% higher in concentration. The "withdrawn" level of 57% is significantly lower than the addicted at 10 min, $p < 0.02$. Control plasma levels of heroin (Fig. 4) dropped at 30 sec, followed by a rise to 52% in 2 min and 72% in 5 min. A steady decrease in heroin levels ensued. In HA rats, plasma concentration ranged between 57%

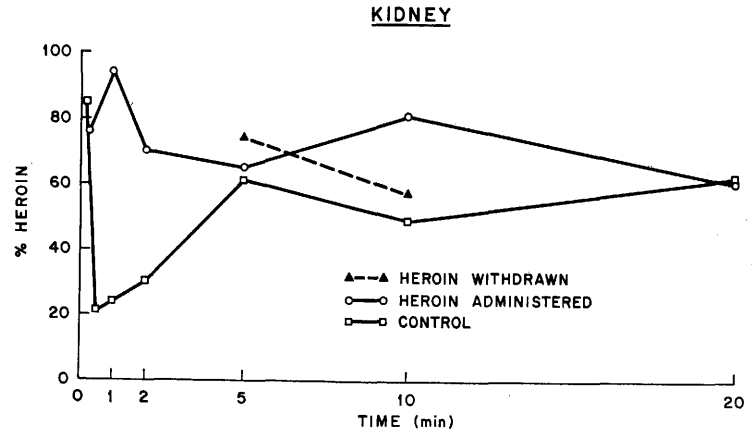

FIG. 3. Concentration of heroin present in *kidney* as percentage of total radioactivity (^{3}H-heroin + ^{3}H-MAM + ^{3}H-morphine) after injection of ^{3}H-heroin. Comparison of control (□), heroin administered (○), and heroin withdrawn (▲).

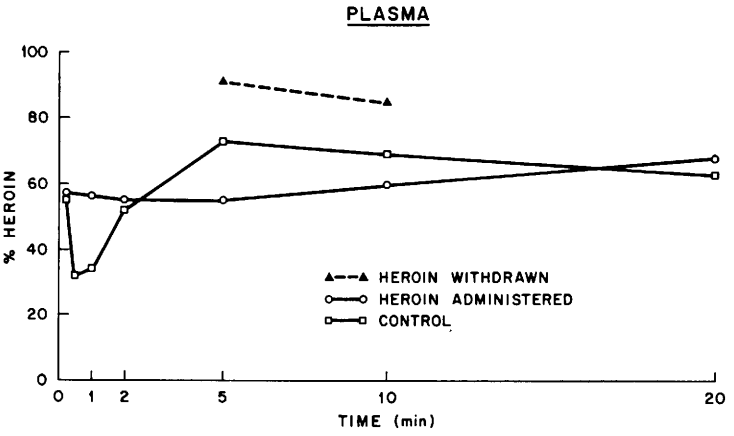

FIG. 4. Concentration of heroin present in *plasma* as percent of total radioactivity (^{3}H-heroin + ^{3}H-MAM + ^{3}H-morphine) after injection of ^{3}H-heroin. Comparison of control (□), heroin administered, (○), and heroin withdrawn (▲).

at 15 sec to 55% at 5 min. A slow increase followed. The difference between control and HA at 5 min is probably significant, $p = 0.05$. In no other tissue did the control levels rise above the HA after 30 sec. The levels of heroin in the HW plasma are significantly higher than the HA at 5 and 10 min, $p < 0.001$ and $p < 0.005$, respectively. The HW levels follow a downward slope parallel to the controls, while remaining 16–19% higher in concentration of heroin.

Observing only tissues in control rats, (Figs. 1–4), it is obvious that there is a dramatic transformation of heroin into its metabolic by-products between 15 sec and 2 min, followed by a rise in heroin concentration between 2 to 5 min. Thereafter, a divergence is seen with liver and brain levels rising slightly and plasma decreasing. Kidney concentrations of heroin are lower than in other tissues except at 1 and 2 min.

The brain levels are slowest to reduce in the early time period with only one half of the total drop evident at 1 min. Other tissues were already increased from the lowest point in heroin concentration by 2 min.

In comparison, the tissues in the HA rats (Figs. 1–4) show brain and liver concentrations of heroin above 88% at all times during observation. The difference between these two tissues is never more than 10% at any point. The levels in plasma remain moderate, diverging no more than 14% throughout, not even reaching 60% heroin until 10 min. The kidney levels fluctuate between the lower plasma and higher liver and brain concentrations.

Discussion. The results establish that heroin metabolism differs significantly between experimental (HA and HW) and control animals. The tissues of experimental rats appear to maintain a steady supply of heroin in the early postinjection period of 0–20 min.

Mulé and Woods (9) postulated that the quantity of morphine present in brain of tolerant dogs influences the uptake of newly injected morphine. It is possible that the heroin present in tissues of HA rats is quantitatively blocking the radioactive tracer compound by tying up receptor sites in the tissues as discussed by Cochin and Axelrod (10). The cold material is not measured by the procedure for detection of labeled chemicals.

The pattern of metabolism of heroin in control rats indicates that 5 min are required for the deacetylation system to stabilize in all tissues studied. After 5 min the theoretical loading of binding sites may occur to stabilize the quantity of heroin. The small chemical amount of heroin injected as tracer, with its high volume of distribution (2), probably cannot bind to all available sites from 5 to 20 min. However, it is possible that after total body distribution, heroin is carried away from tissues which do not readily absorb or

accept it (as morphine less easily enters the brain than heroin) (2). The plasma could carry heroin to those tissues which do absorb and deacetylate it readily, *e.g.*, brain, liver and kidney. The plasma levels of radioactivity would then represent heroin enroute to receptor tissues plus MAM and morphine enroute to excretion.

A parallel interpretation of these *in vivo* experiments could be that blood flushes away MAM and morphine. This would leave tissues with an apparently high concentration of heroin as its deacetylation products are quickly removed. The data in Fig. 4 show the intermediate concentrations of heroin in plasma at all times which could represent the role of plasma as a "carrier" to and from all tissues. The fact that plasma levels dip at 1 min in the controls, as in all tissues, again shows that several minutes are required for diffusion of heroin from total body to specific areas where it later concentrates. Binding site blocking is apparently not a factor in control tissues at this time.

Mulé, Redman and Flesher (11) showed that tolerance does not alter the intracellular localization of morphine in brain or liver. It is therefore probable that the site of metabolic activity in control rats is similar to that in HA and HW rats. The data show correlation between HA and HW heroin levels for brain, liver and kidney: all being higher than in controls. The parallelism in slope between rats withdrawn from heroin for 7 wk and controls indicates a very slow return to normal metabolism after exposure to heroin. However, no analogies between rat and man can be made. Further studies are in progress to determine the effect of heroin administration on *in vitro* metabolism.

Summary. Radioactive heroin was injected into rats treated with (cold) heroin (HA), nontreated control rats, and previously treated "withdrawn" rats (HW). The animals were sacrificed between 0–20 min postinjection. Brain, liver, kidneys and plasma were removed and assayed for the presence of heroin in relation to MAM and morphine.

There is a significant difference in heroin metabolism between HA and control rats for all tissues studied. Tissues of HA rats maintain a relatively steady concentration of heroin from 0 to 20 min postinjection. Tissues of control rats exhibit an immediate drop in heroin concentration (0–2 min) before rising to a relatively steady state. Seven weeks after withdrawal from heroin, tissue metabolism is slowly returning toward normal but remains at levels closer to those in HA rats. Concentrations of heroin may be maintained by plasma acting as a carrier of heroin into tissues which deacetylate and plasma removing MAM and morphine out of these tissues for excretion.

Differences in maintenance levels may be due to blocking of binding sites.

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