Tolerance to Morphine Effects on Renal Disposition of Xenobiotics in Mice

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ABSTRACT
Morphine administration (20 mg/kg s.c.) slowed renal elimination of phenol red in mice, raising plasma levels of this dye and reducing its levels in urine. After 9 days of twice daily morphine injections up to 100 mg/kg, an acute 20 mg/kg morphine challenge did not produce analgesia or hypothermia as in naive mice. This multiple dose morphine regimen also induced tolerance to the effects of the narcotic on plasma and urine levels of phenol red. Morphine, 20 mg/kg, reduced plasma p-aminohippurate clearance by 72% in naive mice but only by 56% in tolerant mice. However, reduction of iothalamate clearance after an acute morphine challenge did not show a statistically significant difference between naive and tolerant mice. These findings suggest that tolerance is more readily induced to the effects of narcotics on renal blood flow and/or tubular function than to reduction of glomerular filtration. Tolerance to the acute effects of morphine on phenol red disposition is probably due to lessened response of blood flow or tubular function in chronically dosed mice.

Endogenous and exogenously administered opioids have been shown to have potent effects on renal function (Huidobro-Toro and Huidobro, 1981). Both diuretic and antidiuretic properties have been ascribed to opioids, depending on dose, species and state of hydration (Marchand, 1970). Opioids have been suggested to exert renal effects by acting centrally or directly at receptors in the kidney, by nervous mechanisms (Humphreys et al., 1985) and through release of vasopressin (DeBodo, 1944). Some of these effects occur immediately after opioid administration, whereas others develop more slowly. Tolerance has been claimed to certain renal opioid effects, such as to depressed endogenous solute transport (Marchand and Denis, 1968). However, no studies have been reported on tolerance to opioid effects on renal disposition of xenobiotics. We have shown earlier that morphine reduces renal clearance of the anionic dye, phenol red (Hurwitz, 1981). In the present study we evaluated the effects of chronic morphine administration on the disposition of i.v. injected phenol red and on the determinants of dye elimination, i.e., renal blood flow and tubular secretion or glomerular filtration.

Methods

Male Swiss-Webster White mice weighing 25 to 35 g were used in all studies. They were housed at an ambient temperature of 23–25°C with a 12-h light-dark cycle and were allowed food and water ad libitum. To induce tolerance, mice were injected s.c. twice each day with ascending doses of morphine sulfate. Each of the two daily doses was 20 mg/kg for 2 days, 50 mg/kg the next 2 days and 100 mg/kg for an additional 5 days. Control animals were given saline. Tolerance to the chronic regimen was evaluated by response to an acute morphine challenge at 12 to 14 hr after the last of the multiple morphine or saline doses.

When studying phenol red disposition, blood and urine were obtained. For hydration, 5 ml/kg of water was administered by gastric tube. To collect urine, the external urethra was then ligated under light ether anesthesia (Becker and Gibson, 1967) just before the acute challenge with morphine or saline. Thirty minutes later, phenol red, 100 mg/kg (20 mg/ml), was administered into a lateral tail vein. After an additional 30 min, at 1 hr after acute morphine or saline, blood and urine samples were collected into microhematocrit tubes by orbital sinus puncture. The mice were then sacrificed by cervical dislocation, their abdomens opened and bladder contents were aspirated through a needle and transferred into graduated centrifuge tubes. These experiments were ended and urine samples were collected at 30 min as prolonged urethral obstruction would have caused renal failure (Becker and Gibson, 1967). Phenol red, sodium salt (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% sodium chloride and filtered through Whatman No. 1 paper. This dye was measured in plasma and urine samples after dilution in 0.1 N sodium hydroxide and read at 560 nm in a Gilford model 300 N spectrophotometer.

Renal plasma flow was estimated by the clearance of PAH from plasma (Rosenbaum et al., 1973). PAH was measured in 50-μl aliquots of plasma by a modification of the diazotization method of Bratton and Marshall (1939). Because of the plasma volumes needed for PAH assay, only four samples could be obtained from each mouse. Glomerular...
filtration rate was determined by the plasma clearance of iothalamate (Guesry et al., 1975). Meglumine iothalamate (Conray-60, Mallinckrodt, St. Louis, MO) was given into a lateral tail vein and measured spectrophotometrically at 245 nm in 10-μl aliquots of plasma by the method of Medzihradsky et al. (1975).

Body temperatures were measured with a rectal probe attached to a YSI model 73A thermometer. Analgesia was determined by the tail-flick method which was adapted to mice (Janssen et al., 1963). Mouse tails were immersed in water at 52°C. If no response occurred by 30 sec the mouse was released and latency time was recorded as 30 sec.

Plasma PAH kinetics were analyzed monoeXponentially for each animal. The logarithms of the four plasma PAH concentrations vs. time were fit linearly by least-squares. These data were extrapolated to obtain levels at zero time (C0) and elimination constants (k) were derived from the slopes.

Clearances (CI) for PAH were then calculated for each animal thus:

\[
CI = \frac{k \times \text{dose}}{C_0}
\]

Iothalamate kinetics were fit biexponentially for each animal by the CSTRIP method of Sedman and Wagner (1976). Area under the plasma concentration-time curve (AUC) of the nine data points was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982), with the area beyond the last time (t) determined by:

\[
\text{AUC}_{t+} = C_t/k_t
\]

Clearance was then calculated thus:

\[
CI = \frac{\text{Dose}}{\text{AUC}_{t+}}
\]

Data were analyzed for statistically significant differences by analysis of variance and Duncan’s test (Zar, 1984).

Results

Chronic administration of ascending doses of morphine induced tolerance to the analgesic and hypothermic effects of an acute dose of morphine (fig. 1). Tolerant animals had lower body temperatures at 12 hr after the last 100-mg/kg dose of morphine, but an additional 20 mg/kg of morphine caused no further temperature reduction as it did in naive mice. Tail-flick latency was identical at 12 hr after the last of the repeated saline or morphine injections. However, an acute morphine injection was much less effective in producing analgesia after chronic morphine than after saline (fig. 1).

Chronic morphine suppressed the changes in phenol red disposition caused by an acute 20-mg/kg dose of morphine. In these mice, made tolerant by chronic morphine, the acute narcotic challenge did not elevate plasma phenol red levels or lower its excretion into urine, as occurred in naive animals (fig. 2). PAH plasma levels and calculated plasma clearances were the same in morphine-tolerant and naive mice. An acute challenge with 20 mg/kg of morphine to naive mice elevated PAH plasma levels markedly and reduced its clearance by over 70% (fig. 3). When tolerant mice were challenged acutely with this dose of morphine, PAH clearance was less than in saline-challenged controls. However, PAH clearance in morphine-challenged tolerant mice was more than twice the rate in naive mice given morphine acutely. Iothalamate clearance was somewhat greater in tolerant mice than in controls at 12 hr after the last 100-mg/kg dose of morphine (fig. 4). The reduction in iothalamate clearance in response to an acute dose of morphine, 20 mg/kg, was not attenuated by prior morphine treatment.

Discussion

The effects of morphine and other opioids on renal function have been investigated extensively for over half a century (Klemt, 1934). Numerous reports of studies in several species, including humans, have failed to agree on the renal effects of opioids, much less on the mechanisms causing these effects (Fujimoto, 1971). Acutely administered opioids have been shown both to reduce or enhance urine output. Centrally and peripherally located receptors have been implicated, as have mu and kappa subtypes. Mu receptors may reduce urine flow whereas kappa receptor activation is claimed to cause diuresis (Leander, 1983). Morphine has been shown to activate both opioid receptor subtypes in mice (Rathbun et al., 1983). Vasopressin release has been implicated in opioid-induced antidiuresis (DeBodo, 1944; Firemark and Weitzman, 1979; Haldar, 1982), but some investigators have actually claimed that opioids
Fig. 2. For 9 days mice were injected s.c. twice daily with saline (SAL) or ascending doses of morphine (MOR). At 12 to 14 hr after the last injection, they were anesthetized with ether, external urethras were ligated, 5 ml/kg of water was given by gastric tube and SAL or MOR 20 mg/kg, was given s.c. Thirty minutes later phenol red, 100 mg/kg (20 mg/ml) was given i.v. Blood and urine samples were obtained 30 min later. Data are means ± S.E.M. N = 11-13 in each group. *P < .05 compared to acute saline challenge. tP < .05 compared to chronic SAL.

Tolerance to nearly all opioid actions has been sought and

usually easily shown (Adler and Geller, 1984). The renal effects of chronic opioid administration have been investigated extensively and, as with the acute effects, much disagreement remains. Tolerance has been shown repeatedly to develop to the antidiuretic effect of morphine (Marchand and Denis, 1968; Marchand, 1970; Inturrisi and Fujimoto, 1968; Huidobro, 1978; Huidobro-Toro, 1980), but not to its diuretic effect, which has been ascribed to kappa receptor activity. In fact, chronically administered morphine has been claimed to cause diuresis. Chronic morphine administration prevents the reflex increase in cation excretion by the remaining kidney which occurs after acute contralateral nephrectomy (Ribstein and Humphreys, 1983). This observation is one piece of evidence suggesting opioid receptor involvement in the regulation of tubular cation secretion.

In view of their complexity and the near impossibility of fitting disparate reports of renal effects of opioids into a well accepted, consistent framework, we will focus on our own data. Thereby, we intend to avoid having to account for variability in species, dosage regimen, state of hydration, etc. Furthermore, very few of the aforementioned studies explored morphine suppress vasopressin output acutely (Van Wimersma-Greidanus et al., 1979; Christensen and Fjalland, 1982; Leander, 1983). Hypotension after opioid administration has been suggested as the cause of impaired renal function (Bidwai et al., 1975; Haldar, 1982; Wilson and Ngsee, 1982), but this mechanism has also been refuted (Inturrisi and Fujimoto, 1968; Grell et al., 1985; Walker and Murphy, 1984; Kanjanapothi, 1975). Glomerular filtration has been shown to remain constant (Handley and Keller, 1950; Bidwai et al., 1975; Walker and Murphy, 1984) or to be reduced (Handley and Keller, 1950; Huidobro and Huidobro-Toro, 1979; Huidobro et al., 1979; Hurwitz 1981) after morphine. Tubular effects of opioids have also been suggested (Handley and Keller, 1950; Huidobro-Toro and Huidobro, 1981; Huidobro et al., 1981; Walker and Murphy, 1984) or rejected (Inturrisi and Fujimoto, 1968).
plasma clearance of iothalamate after morphine challenge was described in the text. Data are means ± S.E.M. N = 5–8 in each group. *P < .05 compared to acute saline challenge. **P < .05 compared to chronic SAL treatment.

actions on the renal disposition of xenobiotics in vivo. We had shown that opioids cause a dose-dependent reduction in phenol red clearance in mice (Hurwitz, 1981). This morphine effect was reversed by naloxone and was not due to changes in urine pH. Phenol red retention was correlated with morphine-induced reduction of PAH and iothalamate clearance, consistent with impairment of renal blood flow and glomerular filtration. The present study confirms these acute effects and defines selective tolerance to these indicators of renal function.

Our chronic (multiple injection) dosing regimen caused tolerance to opioid-induced analgesia and hypothermia. Inasmuch as tolerance to all narcotic effects does not develop synchronously, this finding does not assure renal tolerance. Figure 2 shows that the chronic morphine regimen we chose did attenuate the enhanced plasma retention and reduced urinary elimination of phenol red after an acute morphine challenge. Plasma levels of PAH were affected similarly by chronic narcotic administration. Acute morphine challenge raised PAH levels less in tolerant mice than in naive animals (fig. 3). In contrast, plasma clearance of iothalamate after morphine challenge was similar in tolerant and naive mice. In fact, because iothalamate clearance was somewhat enhanced in tolerant mice (fig. 4), the relative reduction in clearance due to an acute morphine challenge was greater in tolerant (50%) than in naive mice (39%). Thus, the chronic morphine regimen, which induced tolerance to the acute effects of morphine on disposition of phenol red and PAH, failed to induce a similar response to iothalamate. Phenol red and PAH clearances are determined mainly by renal blood flow and anion secretion in the proximal tubule and less by back diffusion and protein binding. Iothalamate is cleared by glomerular filtration. Our findings suggest that tolerance develops to the effect of morphine on renal blood flow and/or proximal tubule function, whereas tolerance to the opioid effect on glomerular filtration is more difficult to induce. Other investigators (Huidobro and Huidobro-Toro, 1979; Huidobro et al., 1979), have shown, in rats, that chronic morphine administration does induce tolerance to reduction in creatinine clearance caused by an acute dose of narcotic. Thus, under different conditions, tolerance to opioid-induced reduction in glomerular filtration has been shown in vivo.

Earlier reports have described impaired PAH uptake into kidney slices from morphine-treated rodents (Marchand et al., 1969; Berndt and Ho, 1979). In these studies, although tolerance developed to this morphine effect, acute morphine administration caused no reduction in tissue uptake of PAH, unlike our findings in vivo. Thus, it is difficult to extrapolate from the results in vitro to our current observations. Nevertheless, tolerance in both the in vitro experiments and in our studies in vivo corroborate involvement of opioid receptors as was suggested by naloxone reversal (Hurwitz, 1981). Tolerance, a phenomenon characteristic with opioids, also provides evidence against competition by morphine or its metabolites for the anion secretion sites in the proximal tubule, to which tolerance has never been described. Although vasopressin may mediate some of the actions of opioids on urine output by increasing distal tubule permeability, it evidently plays no role in the renal effects of morphine which we have shown on xenobiotic disposition, which result from changes in glomerular filtration, renal blood flow and/or proximal tubule function.

The present studies confirm our earlier findings of acute opioid effects on renal disposition of xenobiotics in vivo. Tolerance to some of these actions of morphine is demonstrated, which suggests that impaired phenol red elimination after morphine was mediated by modified tubular secretion or renal blood flow rather than by reduced glomerular filtration.

References


