The quantitative determination of codeine in the remaining extract was by g.l.c. on Carlo Erba, series 4200, equipped with a 2 m × 4 mm glass column packed with 1% OV 17 on anakrom ABS 90–100 mesh, and flame ionization detector. Temperatures for injector, column and detector were 300 °C, 260 °C, and 320 °C respectively. The internal standard (orphenadrine) was previously added to urines, before the hydrolysis, as it does not interfere. Calibration was by use of the codeine/orphenadrine ratio with various concentrations of codeine (0·1–2·0 μg ml^{-1}). The detection limit for codeine was 0·1 μg ml^{-1} by both t.l.c. and g.l.c. The presence of codeine was confirmed by mass-spectrometry on an LKB series 2091: temperatures of ion source and separator were 250 °C and 230 °C respectively; electron energy 20 eV.

Results

Morphine was detected and quantitized in all urine samples. On the other hand codeine was *not present* in urine samples of group I and *present* in those of group II (heroin addicts or users). The amount of codeine in these urine samples was between 4 to 18% of the morphine concentration.

Discussion

This study, in agreement with Yeh's and Yong's observations, has shown that biotransformation of morphine to codeine via *O*-methylation does not occur in man. None of the urine samples of the 70 subjects receiving morphine only (60 to 120 mg daily) contained codeine (the codeine impurity in Italian pharmaceutical morphine HCl is 0·12 to 0·25% and therefore non-detectable with the techniques used). Codeine was present in all urine samples of 150 heroin addicts as a metabolite of acetylcodeine which is an impurity in illicit heroin (in Italy illicit heroin seizures contain 5 to 15% acetylcodeine relative to heroin). During 1980 in our laboratory, of 3500 urine samples from various sources analysed for drugs of abuse (opiates), 65% contained morphine and codeine and always involved heroin addicts or users.

Our findings suggest that it may be possible to differentiate heroin or morphine consumption by the presence of codeine and morphine in urine or in biological specimens if they are in amounts corresponding in our research.

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Morphine antidiuresis in the rat: biphasic effect of the opiate on the excretion of urine electrolytes

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We have recently shown that although both morphine and vasopressin decrease urine outflow in the rat, they have differential effects on the excretion of urinary electrolytes. Morphine, levorphanol, (−)-methadone and the novel opioid-like peptides reduce urine outflow and produce a hypotonic urine characterized by a low concentration of Na^+, K^+ and Cl^-, while the exogenous administration of vasopressin produces antidiuresis with a markedly hypertonic urine due to the large reabsorption of water (for a review see Hays 1980; Huidobro et al 1979; Huidobro & Huidobro-Toro 1979; Huidobro-Toro et al 1979; Huidobro-Toro & Huidobro 1981). When vasopressin is given with morphine the effect is not additive, but rather morphine antagonized the effect of vasopressin on the excretion of urinary electrolytes (Huidobro & Huidobro-Toro 1979), suggesting that the release of antidiuretic hormone may not be the major determinant of the morphine antidiuresis as proposed by de Bodo (1944). The recent finding that Brattleboro rats (animals with severe diabetes insipidus) respond to the full antidiuretic effect of morphine or β-endorphin regardless of their genetic lack of antidiuretic hormone further supports this idea (Huidobro-Toro 1980). Our aim has been to gain further information on the possible involvement of the antidiuretic hormone on the morphine antidiuresis by focusing on the effect of different doses of morphine on urine outflow and electrolyte concentrations in the rat.

Groups of eight adult Sprague Dawley rats (200 ± 15 g)

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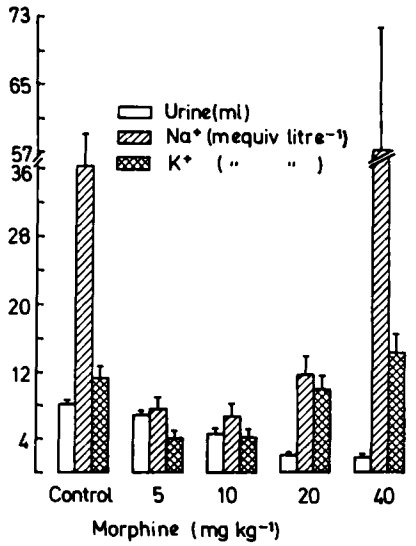

FIG. 1. Dose-response curve of the morphine antidiuresis and its effect on the concentration of urine electrolytes. Morphine as the hydrochloride salt was injected i.p. 10 min before the oral administration of 0·5% NaCl; the control group was injected with 1 ml kg^{-1} saline otherwise treated identically. Results are expressed as the mean output of urine (ml) collected during the 80 min after morphine or saline. Sodium or potassium as mequiv $litre^{-1}$ determined in the specimens obtained during the 60–80 min period following drug treatment. Columns indicate the mean value, bars the s.e. Open columns, urine. Hatched columns Na^+. Cross-hatched columns K^+.

from the strain bred at the University Institute were housed at 22 ± 1 °C, with artificial light from 6:00–18:00 h. Rats were fasted 18 h before the experiment, but had free access to tap water. To increase diuresis and to study the antidiuretic effect of morphine, 25 ml kg^{-1} tap water was administered orally followed 1 h later by oral sodium chloride (50 ml kg^{-1} 0·5%) as previously described by Huidobro & Huidobro-Toro (1979). Urine was collected and measured 60 and 80 min after the treatment with either morphine or saline i.p. Injections of morphine were given as described by Huidobro & Huidobro-Toro (1979). Urine electrolyte concentrations were determined by flame photometry. The results are expressed as the mean ± s.e. of each variable. The two tail Student's *t*-test was used to compare the effect of morphine the saline control. Significance was set at $P < 0.01$.

Morphine caused a dose-dependent reduction in the outflow of urine. A dose of 10 mg kg^{-1} decreased the urine volume measured 80 min after treatment by 40% ($P < 0.01$), 40 mg kg^{-1} by about 80% and 60 mg kg^{-1} caused anuria (Fig. 1).

Morphine antidiuresis was associated with a reduced excretion of Na^+ and K^+. Morphine (5 mg kg^{-1}) decreased urine output by 15% ($P > 0.10$) but significantly lowered the concentration of urine electrolytes ($P < 0.01$) (Fig. 1), while 10 mg kg^{-1} caused a more profound antidiuresis coupled with an 81% decrease in Na^+ concentration and a 63% reduction in urine K^+ ($P < 0.01$). The antinatriuretic and antikaliuretic effect of morphine was biphasic since 40 mg kg^{-1} produced the most severe oliguria while markedly increasing the urine Na^+ and K^+ concentration compared with the effect of 10 or 20 mg kg^{-1} morphine or with the saline-treated controls (Fig. 1). Although 20 mg kg^{-1} morphine produced as much antidiuresis as 40 mg kg^{-1}, the latter dose produced a marked increase in the urine Na^+ and K^+ ($P < 0.05$).

These results suggest that at least two different mechanisms are involved in the action of opiates on urine production and composition. Doses of morphine between 5–10 mg kg^{-1} i.p. cause oliguria and a hypotonic urine with low concentrations of both Na^+ and K^+. We previously demonstrated that the reduced excretion of electrolytes by the lower doses of morphine is associated with a significant increase in the corresponding plasma concentrations of electrolytes (Huidobro et al 1979). The present results indicate that larger doses of morphine produce a profound decrease in the urine outflow associated with a rise in the excretion of urine Na^+ and K^+. The mechanism of antidiuresis and hyponatruresis and hypokaliuresis caused by low doses of morphine is not clear. Because morphine at low doses reduces rather than increases the urine electrolyte concentrations, these data do not support vasopressin as mediating the urinary effects. We have shown that the acute injection of 7·5 mg kg^{-1} morphine reduced renal clearance of endogenous creatinine by about 50% (Huidobro et al 1979), so it is possible that morphine causes a reduction in the rate of glomerular filtration via a central and/or peripheral opiate mechanism and that the cardiovascular effect of morphine does predominate over other effects at the lower range of morphine concentrations. At the higher concentrations it is likely that morphine may cause release of antidiuretic hormone from the hypophysis, this effect is not evident with the lower doses.

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