

## **MORPHINE TRANSPORT IN THE ISOLATED PERFUSED RAT KIDNEY**

**P J Ratcliffe, J W Sear, C W Hand, R A Moore**

*John Radcliffe Hospital, Oxford, United Kingdom*

### **Summary**

Morphine handling has been studied in the isolated perfused rat kidney. Active secretion of morphine was demonstrated which was dose-dependent over a perfusate concentration range similar to the therapeutic concentration used in man (60–640ng/L). Very high secretory rates resulted in morphine clearance greatly exceeding glomerular filtration rate. In the rat the kidney plays a major role in the elimination of morphine.

### **Introduction**

Despite reports in the Nineteenth Century of the danger of prescribing opium in Bright's disease [1], the view has arisen that the liver rather than the kidney is the major site of morphine metabolism. This view has recently been challenged. Firstly morphine metabolism was found to be normal in patients with cirrhosis of the liver [2] and secondly, prolonged action and delayed elimination of the drug have been reported in renal failure from a variety of causes [3]. Although it is recognized that species differences in morphine metabolism may exist, these clinical studies implicating the kidney in morphine elimination in man are backed by experimental studies in animals. Transport and metabolism of morphine has been demonstrated in isolated non-perfused proximal tubular segments of rabbit kidney [4] and secretion and metabolism of morphine have been shown in the chicken kidney after infusion of morphine into the renal portal vein [5]. Although these studies demonstrate that the kidney in isolation may eliminate morphine they do not quantify the renal contribution to morphine clearance at therapeutic concentrations of the drug. This has been investigated using the isolated perfused rat kidney.

### **Methods**

Isolated kidneys from male Wistar rats (330–400g) were perfused with a blood-free, recirculating medium consisting of 6.7 per cent bovine serum albumin in

Krebs-Henseleit buffer gassed with 95 per cent O<sub>2</sub>: 5 per cent CO<sub>2</sub>. Glucose (5mmol/L) and all 20 physiological aminoacids were added as substrate in concentrations routinely used in this laboratory [6]. Glomerular filtration was measured by clearance of C<sup>14</sup> inulin (Cin). Morphine was added to the perfusate as morphine sulphate pentahydrate. After a 30 minute equilibration period urinary collections were made every 15 minutes. The duration and perfusion experiments varied from 60 to 180 minutes. Morphine clearance (C<sub>morph</sub>) and net tubular morphine transport were calculated by standard urinary clearance techniques after the measurement of the effective filtration co-efficient for morphine from the ratio C<sub>morph</sub>/Cin during perfusion with iodoacetamide (6mmol/L) which non-selectively inhibits active tubular transport. Morphine concentrations were assayed by radioimmunoassay using two antisera: rabbit anti-morphine antiserum produced by haptenization to the three position of the molecule (Guildhay antisera, Surrey) and antiserum produced by linkage to the two position. The radioimmunoassay procedure is described fully elsewhere [7]. Results are expressed as mean±1 standard deviation.

## Results

Initially high fractional reabsorption of sodium (FrNa<sup>+</sup>) of 99.32±0.32 per cent and clearance of inulin of 0.65±0.15ml/min/g wet weight were well maintained

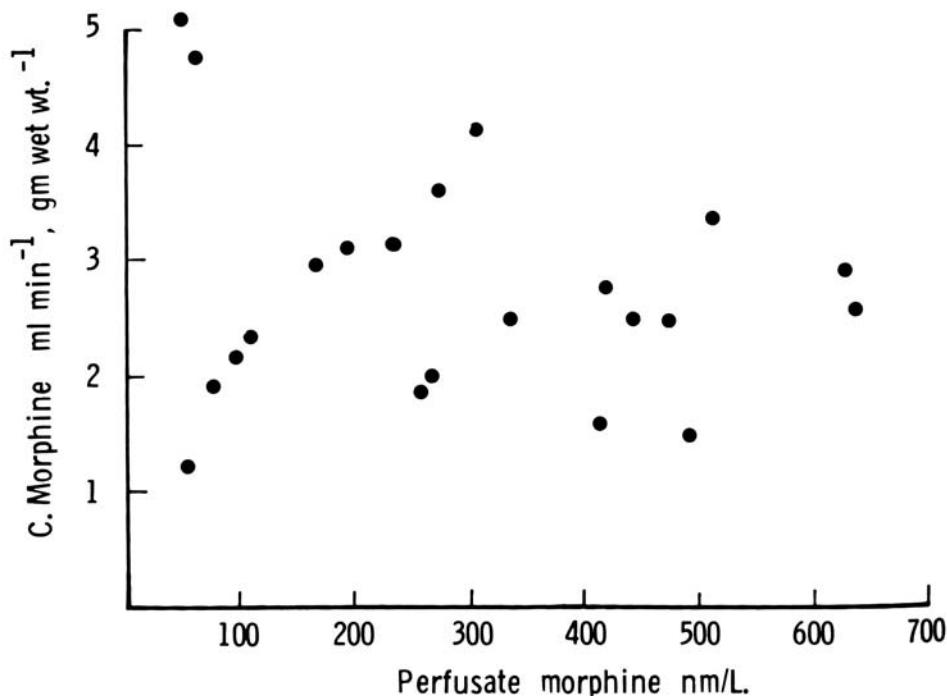


Figure 1. Urinary morphine clearance rates in the first 30 minutes of perfusion after equilibration

in perfusion experiments lasting up to three hours. After addition of iodoacetamide to the perfusate Cin fell to  $0.46 \pm 0.13$  ml/min/g wet weight,  $FrNa^+$  fell to  $8.0 \pm 2.2$  per cent and the ratio  $C_{morph}/C_{in}$  fell to  $0.61 \pm 0.08$ . This value is used as the effective glomerular filtration coefficient for morphine in calculation of net tubular transport of morphine.

In all normal perfusions, during the first 30 minute period after equilibration,  $C_{morph}$  was very much higher than  $C_{in}$  ( $C_{morph}/C_{in} = 4.41 \pm 2.61$ ). These very high rates of morphine clearance were seen over the entire range of perfusate concentrations studied (Figure 1). Net transtubular transport rates are shown in Figure 2. The very high transport rate is dose dependent and no evidence of saturation was seen in this dose range. Considerable variability in transport rates was noted between animals.

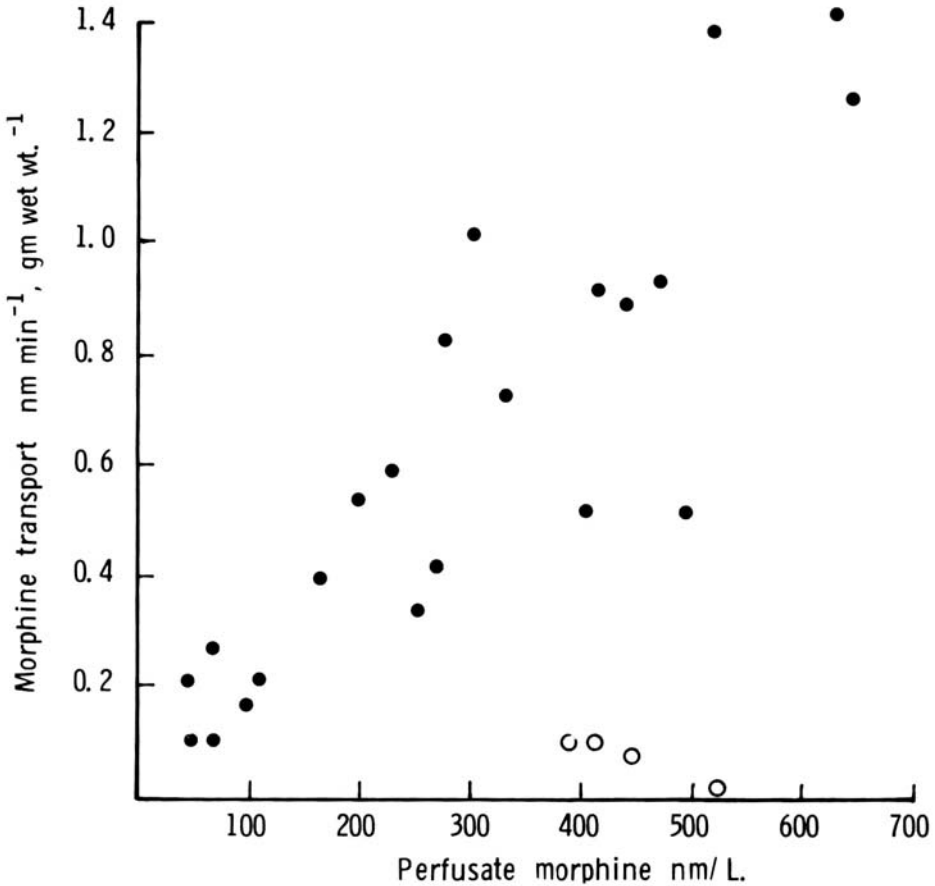


Figure 2. Net tubular morphine transport from perfusate to urine in the first 30 minutes of perfusion after equilibration: ● no inhibitor; ○ after addition of cyanin dye No. 863

Initially high net transport rates for morphine were not well maintained during perfusion. In three 3-hour experiments, morphine clearance declined from  $3.0 \pm 0.80$  ml/min/g wet weight, to  $0.67 \pm 0.4$  ml/min/g wet weight (Figure 3) even though perfusate concentrations remained in the range studied in the shorter experiments.

Morphine transport was inhibited by the organic cation transport inhibitor 1 ethyl-3,6-dimethyl-2-phenyl, 4 pyrimido-2' cyanine (Cyanine Dye No 683 Uniscience Limited). Addition of  $200 \mu\text{g}$  inhibitor to the 100ml perfusate almost completely abolished morphine transport so that for a perfusate morphine concentration of 400–500 nmol/L, transport fell from 500–930 pmol/min/g wet weight to 15–104 pmol/min/g wet weight. At this concentration of inhibitor there was no change in Cin or FrNa<sup>+</sup>.

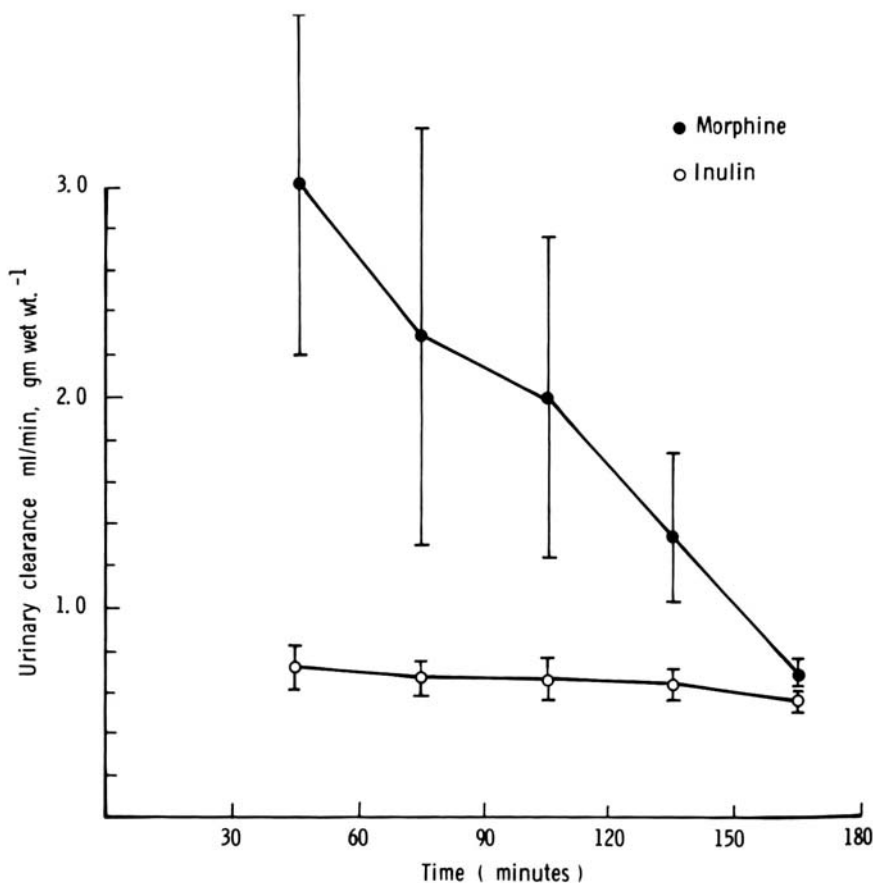


Figure 3. Changes in the clearance rates of morphine and inulin during 3-hour perfusion experiments

Identical values for perfusate and urinary morphine concentration were obtained with each of the two antibodies in nine perfusion experiments. In the remaining experiments assays were performed only with the antibody conjugated through the two position.

## Discussion

The perfused kidney has been used in this study to assess the role of the kidney in isolation in the metabolism and clearance of morphine. Detailed data are available in this model for the tubular transport of many substances and demonstrate that proximal tubular functions such as transport of sodium, glucose, low molecular weight proteins and para-amino-hippuric acid are very well preserved [8]. The data obtained here for morphine are therefore likely to be an accurate reflection of morphine handling in vivo.

Perfusion with 6mmol/L iodoacetamide leads to virtually complete inhibition of active tubular transport but at this concentration has little effect on glomerular filtration [9]. The ratio  $C_{morph}/C_{in}$  of  $0.61 \pm 0.08$  observed under these conditions is most likely to represent hindrance to the glomerular filtration of morphine by binding to bovine serum albumin. During normal perfusion  $C_{morph}/C_{in}$  was very much higher reflecting a high rate of net transtubular transport of morphine from perfusate to urine. Such transport is not necessarily active as ionic trapping of a weakly basic substance such as morphine, in urine more acidic than perfusate could give rise to net transtubular movement by passive diffusion of the unionized species. However, during perfusion under these conditions the urine to perfusate pH gradient is small, being of the order of 0.5pH units, and cannot account for the very high urine to perfusate concentration gradients achieved for morphine which in some experiments exceeded 200:1. Further evidence of an active secretory system for morphine was provided by the effect of cyanine dye No. 863. The substance inhibits the transport of tetraethylammonium and N<sup>1</sup>methyl nicotinamide and also inhibits morphine secretion and metabolism to morphine ethereal sulphate in the chicken kidney [5,10]. It is thought to have a specific action on a transport system for organic cations located on the basolateral membrane of the proximal tubular cells. The present experiments demonstrate the presence of such a system in the rat and show that it is capable of high rates of morphine secretion at therapeutic perfusate concentrations.

The two antibodies used for the assay of morphine in this study have been tested for cross reactivity with morphine-3-glucuronide. The antibody linked through the three position showed 100 per cent cross reactivity with morphine-3-glucuronide whereas the antibody linked through the two position showed no cross reactivity. The identical results obtained with each antibody indicate that there is no metabolism to morphine-3-glucuronide. Metabolism of morphine to other metabolites is not excluded by this study.

Whether or not metabolism is occurring in addition to secretion, the high clearance of morphine achieved is such that the kidney must play an important and most probably the major role in elimination of this drug in the rat.

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