Biochemical Pharmacology, Vol. 25, pp. 1271–1284. Pergamon Press, 1976. Printed in Great Britain.

FACTORS AFFECTING MORPHINE UPTAKE INTO KIDNEY SLICES

DAVID N. TELLER*, TERESITA DEGUZMAN and ABEL LAJTHA

New York State Research Institute for Neurochemistry and Drug Addiction, Ward's Island, New York, N.Y. 10035, U.S.A.

(*Received* 25 *April* 1975; *accepted* 8 *August* 1975)

Abstract—The uptake of [*n*-methyl^{14}C]morphine by mouse kidney slices was saturable, reversible, temperature- and pH-dependent, and was inhibited by strong metabolic poisons and by structural analogs, thereby satisfying criteria for mediation by active transport processes. There were species differences: rat kidney cortex slices took morphine up at rates similar to those of mouse kidney, but guinea pig kidney cortex had lower uptake. Thin-layer chromatography (t.l.c.) of slice extracts after incubation with [^{14}C]morphine did not indicate significant metabolism of morphine. Morphine efflux after equilibrium uptake from 30 nM or 10 μM initial medium levels required 20 min for 50 per cent of the [^{14}C]morphine to exit. The uptake at 5 min was proportional to the equilibrium level at 30 min from 5 nM to 10 μM. No evidence for counter-transport was observed. Tissue/medium levels of 10–12 at 39–90 nM morphine (after 30 min at 37°) were reduced 50 per cent at pH 6·5 or at 20°, or by mitochondrial enzyme inhibitors, e.g. rotenone. Narcotic antagonists and analogs (methadone, nalorphine and levorphanol) and quinine also reduced the uptake of morphine from medium levels of 0·01 to 0·1 μM. However, at morphine concentrations above 10 μM, narcotic analogs or antagonists up to 50 μM did not inhibit uptake. Transport system inhibitors, quarternary bases, reducing agents and SH-oxidants also inhibited morphine uptake from 30 nM to 0·5 mM. Phloretin, phloridzin and atractyloside did not block uptake, while glucose, ouabain and NaF were very weak inhibitors. The results suggest that uptake of morphine in kidney slices involves SH groups and mitochondrial activity rather than glycolytic or ion-pump mechanisms. With a few exceptions, the characteristics and inhibitor sensitivity of morphine uptake by kidney slices and of amino acid uptake by brain slices appear similar.

The major excretory route for morphine is via renal transport [1–3]. Active transport of morphine into kidney slices from rats was shown by Bell [4] to be energy dependent, pH sensitive, and saturable. Subsequently, Hug [5] extended these findings to dihydromorphine and related narcotics using dog kidney cortex. As part of a program to determine whether specific transport processes exist for drugs of abuse into brain tissue (see preliminary reports [6, 7]), we used kidney tissues from the same mice, rats and guinea pigs as positive controls for the various attempts to modify brain uptake of the drugs. Although we were not able to demonstrate that active transport of morphine occurs in rodent brain slices [7, 8], we confirmed the presence of transport processes for morphine in kidney slices. In contrast, amino acids are actively transported to higher tissue concentrations by brain slices than they are in kidney tissue slices, and we could study effects of various experimental treatments on the transport characteristics of both tissues. Results from the kidney slice experiments indicated that a major portion of the energy for morphine uptake was due to mitochondrial activity [9]. These observations were extended to studies of the energetics of active transport of amino acids in both kidney and brain slices [10]. Here we report studies of factors affecting morphine uptake into kidney tissue that seem to be remarkably similar to those controlling the active transport of amino acids into kidney or brain slices.

MATERIAL AND METHODS

Materials. Morphine (57 mCi/m-mole), labeled with ^{14}C in the *N*-methyl group, was obtained from Amersham–Searle. Morphine hemisulfate, from Mallinckrodt Chemical Works, was converted to the hydrochloride by reaction with $BaCl_2$ and CO_2. The solution was dried under N_2; the morphine HCl was recrystallized from ethanol and was assayed colorimetrically with the same reagents and procedure used for protein [4, 8] with authentic morphine hemisulfate as a standard. Procedures for calibration of volumetric glassware and liquid scintillation spectrometry were reported previously [8]. Radioactive amino acids, uniformly labeled with ^{14}C, were obtained from New England Nuclear Corp. Nonradioactive compounds were prepared in water, buffer, medium, or solvent as indicated, and controls were added to the experimental protocol to measure the effects of the diluent, alone, on the uptake of the radioactive drug. Concentrated solutions of the narcotics and their analogs were kept dark and cold, or frozen, before use. With some drugs, e.g. levorphanol, nalorphine, chlorpromazine and quinine, the room was darkened during the entire procedure to avoid photodecomposition of the dilute compounds. Table 1 lists the various drugs and inhibitors† used in this study; commercial source, analytical data and formula weight used in

* Present address: Dept. of Psychiatry and Behavioral Sciences, Univ. Louisville Medical School, P.O. Box 1055—MDR 517 Louisville, KY 40201, U.S.A.

† In this report, the term inhibitor is used to identify those compounds that decrease morphine uptake into slices, as well as specific inhibitors of enzymes.

calculating concentrations; and the method of solubilization.

Methods. Mice, rats and guinea pigs were inbred from strains derived from Swiss–Webster, Wistar and Cayley varieties respectively. The mice were 6 to 10-weeks-old, the rats were 6 to 8-months-old, and the guinea pigs were 9-months-old when they were used for these experiments. Some animals were injected with morphine, from 40 mg/kg, i.p., to more than 1 g/kg, s.c., to produce tolerance.* Animals 'withdrawn' from morphine were given no injection for 18 hr prior to being killed; 'chronic' animals received a last dose of 400 mg/kg, i.p., 2–3 hr before use; 'acute' animals received a single dose of 40 mg/kg, i.p., 1 hr before they were killed. In all cases, both sexes were used because no consistent effects were observed that might be attributable to sex differences.

Kidneys were removed after the animals were decapitated; the renal tunic was removed; each kidney was rinsed in ice-cold incubation medium and divided longitudinally opposite the hilum. In the case of mouse tissue, the entire half-kidney was sliced transversely, but rat and guinea pig kidneys were cleaned of connective tissue, vessels, and the hard mass of urinary collecting tubules before the cortex and medulla were dissected for slicing. The variations in weight between right and left kidneys, and between males and females were randomized in the distribution of the tissue slices into the incubation flasks.

The methods of the slice preparation, incubation and determination of amino acid uptake, ion content, ATP levels, dry weight and swelling followed the same procedures as have been used in this laboratory for brain slices [8, 9, 11]. Briefly, 0·42-mm thick slices were prepared with a McIlwain–Mickle tissue slicer. After the slices from half of a mouse kidney were incubated in 4·5 to 5 ml of oxygenated medium for 30 min at 37°, the labeled morphine or amino acid was added for the experimental incubation period. The HEPES-2 medium contained 119 mM NaCl, 5·0 mM KCl, 0·75 mM $CaCl_2$, 1·2 mM $MgSO_4$, 1·0 mM NaH_2PO_4, 1·0 mM $NaHCO_3$, 10 mM glucose and 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES); 12 mM NaOH adjusted the pH to 7·35, which increased the Na^+ concentration to 132 meq/l.

* Animals were injected every 12 hr, and the morphine dose/kg was increased daily: 40, 80, 160, 240 mg., i.p., 240 mg, s.c., 320 (3 days), 1 g, s.c. (3 days).

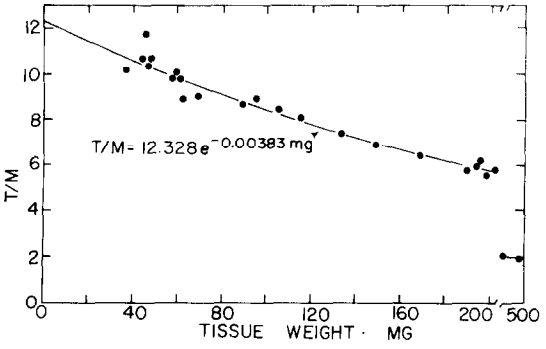


Fig. 1. Uptake of morphine by kidney tissue is dependent upon tissue mass. Slices were equilibrated for 30 min at 37° before the addition of morphine[^{14}C] to a final concentration of $3{\cdot}17 \times 10^{-8}$ M for a 30-min uptake incubation. Varying amounts of tissue were placed in the flasks (from 37 to 500 mg). The minimal T/M, 2·01, was obtained experimentally by incubating 500 mg tissue. A linear fit of the data yields zero T/M at 375 mg; however, a better fit, r (wt/(T/M)) = (−) 0·916, was obtained with 24 points. $T/M = 12{\cdot}328e^{-0{\cdot}00383(mg)}$, using the $y = ae^{bx}$ formula (solid line).

The medium was filtered from the slices after incubation; the tissue was frozen and weighed. In some cases, the frozen tissue was homogenized in 3% perchloric acid (PCA) (w/v) for determination of ions or ATP. In most cases the tissue mass was dissolved in 1 N NaOH at 60° for 10–20 min. This was acidified with HCl and was rinsed into tT76 scintillation fluid with sufficient water to form a thixotropic gel [8]. The tT76 scintillator contained 8 g of 2,5-diphenyloxazole and 150 mg of 2,2'-*p*-phenylenebis(4-methyl-5-phenyloxazole)/l. of toluene, and 860 ml Triton X-100. Corrected dis./min were measured with an Intertechnique/Teledyne SL-30 liquid scintillation spectrometer with a Multimat 8K-bit computer. The counting efficiency for ^{14}C varied from 92 to 78 per cent with a background of 45 cpm.

Calculations of the results were based upon a tissue water content of 0·8 ml/g of fresh weight for both brain and kidney:

$$\frac{\text{(dis./min in tissue/tissue wet weight)} / 0.8}{\text{dis./min/ml medium at the start of incubation}} = \text{T/M}$$

Whenever more than 10 per cent of the radioactivity was removed from the medium by the tissue mass

Table 1. Substances tested for effect on uptake of morphine by kidney slices

Compound	Formula wt	Source	Grade*	Effect†
General metabolic and glucose transport inhibitors				
Ouabain octahydrate	729	Mann	U.S.P.	wI
KCN	65·1	Fisher	Reagent	mI
NaN_3	65·02	Matheson, Coleman & Bell	Reagent	mI
Fluoroacetic acid	78·04	CalBiochem	A	wI
Phloretin‡,§	274·3	K & K	95	0
Phloridzin‡	436·4	K & K	99	0
IAc‖	186	Sigma	98·5	mI
NaF	42	Fisher	Reagent	wI
Dimethyl ketone	58·1	Fisher	Reagent	mI
Ethanol	46·1		95 (vol%)	mI

Table 1—*continued*

Compound	Formula wt	Source	Grade*	Effect†
Bases				
Choline chloride	139·6	Sigma	99%	wI
TEA bromide	210·2	K & K	99	wI
Hexamethonium bromide	362·2	Sigma	98	wI
Decamethonium bromide	418·4	K & K	99	mI
Hemicholinium tribromide	574·4	Aldrich	98	mI
Succinylcholine chloride dihydrate	397·3	Sigma	98	wI
Procyclidine hydrochloride	323·4	Burroughs Wellcome	100	sI
Quinine hemisulfate hydrate from (diquinine dihydrate monosulfurate)	391·5	Sigma	U.S.P.	sI
Narcotic analogs and antagonists				
DL-Methadone hydrochloride	345·9	Mallinckrodt	U.S.P.	sI
Levorphanol-L-tartrate dihydrate	443·5	Roche	98·3	sI
Dextrorphan-*d*-tartrate hydrate	425·5	Roche	99	sI
Nalorphine hydrochloride	347·8	Merck	U.S.P.	sI
Naloxone hydrochloride	363·8	Endo	99	wI
Transport system inhibitors				
Probenecid	285·4	Sigma	99	wI
Amantadine hydrochloride	187·7	Aldrich	100	mI
Atractyloside, potassium	839·0	CalBiochem	A	0
Valinomycin	1111·4	CalBiochem	A	sI
Respiratory uncoupling agents				
2-Nonyl-hydroxyquinoline-*n*-oxide‡	287·4	Sigma	98	sI
Quinacrine dihydrochloride, dihydrate	508·9	Sigma	99	sI
Oligomycin	424, 394	Sigma	(15A, 85B)	sI
Amobarbital	226·3	Sigma	99	wI
Chloramphenicol‡,§	323·1	Sigma	98	wI
Gramicidin D (in HAc)	2000	CalBiochem	95	wI
Oxidative substrates and endogenous amines				
Glucose	180·2	Fisher	Reagent	wI
L-Glutamic acid	147·1	CalBiochem	A	wI
DL-Lactic acid, sodium (syrup)	112·06	Sigma	60	mS
α-Ketoglutaric acid	146·1	Sigma	98	mS
Oxaloacetic acid	132·07	Sigma	100	wI
Pyruvic acid	88·1	Sigma	96	sS
Succinic acid, sodium trihydrate	159·1	Sigma	98	mS
L-Alanine	89·1	CalBiochem	A	0
L-Lysine hydrochloride	182·7	CalBiochem	A	wI
L-Cadaverine, dihydrochloride	175·2	CalBiochem	A	wI
SH-reagents				
DTE	154·3	Pierce	100	mI
NEM	125·1	Mann	100	sI
PCMB, sodium	379·2	CalBiochem	A	mI
CPZ, hydrochloride	355·3	Smith, Kline & French	99	sI
Electron transport (redox) enzyme inhibitors				
2,4-DNP	184·1	K & K	100	mI
Methylene blue	371	Sigma	97	mI
PMS	306·3	DAJAC (Borden)	100	sI
Ascorbic acid	176·1	Fisher	U.S.P.	mI
Antimycin-A, 1‡,§	548·6	CalBiochem	99·5	sI
Rotenone §	394·4	Sigma	92	sI
Malonic acid	104·1	Sigma	98	wI

* Per cent composition where numerical data are shown.

† Key: w = weak (effective at 10^{-2}–10^{-3} M); m = moderate (effective at 10^{-4}–10^{-3} M); s = strong (effective at $< 10^{-5}$ M); I = inhibitor of uptake; and S = stimulator of uptake.

‡ Soluble in alcohol.

§ Soluble in acetone.

‖ Abbreviations: CPZ, 2-chloro, 10-dimethylaminopropyl phenothiazine (chlorpromazine); 2,4-DNP, 2,4-dinitrophenol; DTE, dithioerythritol; IAc, iodoacetic acid; NEM, *n*-ethylmaleimide; PMS, *n*-methylphenazonium methyl sulfate (phenazine methyl sulfate); PCMB, *p*-chloromercuriphenyl sulfonic acid; TEA, tetraethylammonium bromide.

during the incubation, the final medium concentration was determined by counting a sample of the medium from each incubation flask.

Efflux of radioactive morphine was measured by filtering the radioactive medium from the slices through a Hirsch funnel, rinsing with 2 ml of warmed,

oxygenated medium, and transferring the slices to fresh oxygenated medium that contained no radioactivity. After an additional 2–120 min, the slices were filtered from the washout medium and were frozen. In some cases, nonradioactive morphine, naloxone, CN^-, ouabain, and other substances were in the 'washout' medium. During the course of these experiments, one unexpected source of variation was traced to the tissue/medium volume. Uptake was greater with small masses of tissue in large volumes of medium. The relationship is shown in Fig. 1. This contributed a small but significant variation among results obtained with, for example, rat kidney cortex, in comparison to data from the more uniform masses of mouse kidney tissue. To avoid artifacts based on this tissue/medium volume effect, the incubation volume was kept constant at 5 ml in a 25-ml flask, with 80–120 mg tissue slices.

Sodium and potassium concentrations of diluted perchloric acid supernatants were measured with an IL/343 flame photometer (Instrumentation Laboratories). ATP was determined with a du Pont Luminescence Biometer [11].

Extraction and thin-layer chromatography of the radioactive material from tissue. Our modifications of the procedures of Misra and Mulé [12] have been reported elsewhere [8]. In a typical experiment, 50 ml of oxygenated HEPES-2 in a 250-ml flask contained 1 g of rat kidney cortex slices. To this was added 0·5 ml of 0·1 mM morphine containing $1{\cdot}2 \times 10^7$ dis./min. After 30 min at 37° the slices were filtered, frozen, weighed and homogenized in 3% PCA (10 vol./g of tissue) as described previously [8] for brain tissue. A sample of the PCA extract (4 ml) or the homogenate was adjusted to pH 10, treated with salt and phosphate buffer, and extracted with isopropanol in dichlorethane. The recovery of homogenate radioactivity was 96–97 per cent in the organic extract. Up to 93 per cent of the radioactivity applied to the t.l.c. plates was recovered at the R_f of morphine (Fig. 2). Similar results were obtained with extracts from slices of mouse kidney and rat brain.

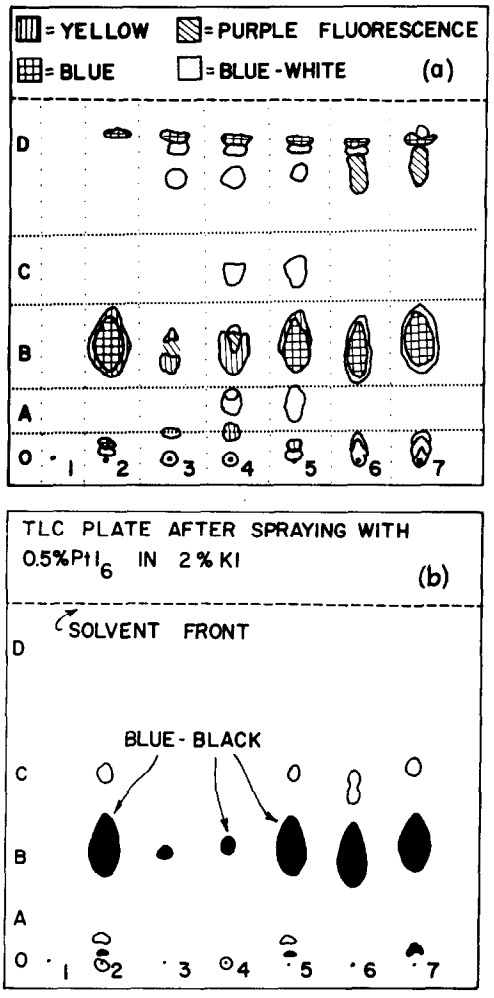


Fig. 2. (a) Indicates the appearance of a developed 250 μM Silica gel G plate under u.v. light. The following extracts were spotted on the marked position: (1) solvent blank from extract of 0·1 m-mole nonradioactive morphine sulfate; (2) extract of carrier (0·1 m-mole) + $1{\cdot}88 \times 10^5$ dis./min of morphine[^{14}C]; (3) extract from rat brain slices, $1{\cdot}5 \times 10^4$ dis./min (no carrier); (4) extract from rat kidney slice homogenate, $3{\cdot}44 \times 10^4$ dis./min (no carrier); (5) same as No. 4 with addition of 0·1 m-mole carrier at start of extraction; (6) extract of a PCA filtrate of mouse kidney slices, $4{\cdot}16 \times 10^4$ dis./min + carrier; (7) same as No. 6 with addition of $1{\cdot}88 \times 10^{-5}$ dis./min of morphine[^{14}C]. (b) Shows the same plate, sprayed with 0·5% PtI_6 in 2% KI. The solvent was ethylacetate–methanol–water–ammonium hydroxide (85:10:3:2). Development time was 2·3 hr at 23°. Recovery of applied dis./min in origin (O) spots was 1·6 per cent and in the area of morphine (sections labeled B) was 82–93 per cent.

RESULTS

Characteristics of morphine uptake into and efflux from kidney slices

Time course of uptake. Uptake was rapid in mouse kidney slices (Fig. 3), and under certain conditions was linear from 2 to 15 min after incubation started.

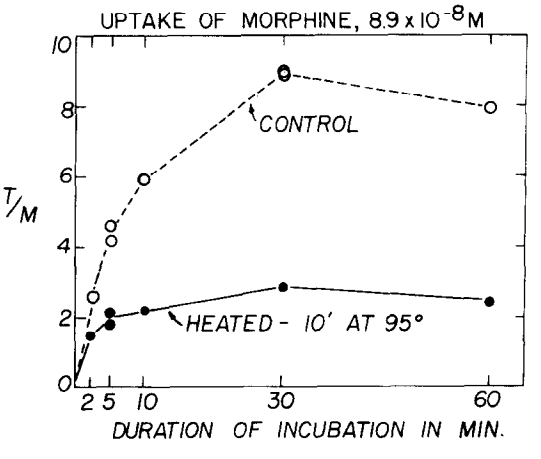


Fig. 3. Time course of morphine uptake by kidney slices. Mouse kidney slices 416 μm thick were suspended in HEPES-2 in ice-water (control) or in a boiling water bath. The temperature of the medium in the heated test tubes rose to 95° within 1 min, and 10 min later the tubes were cooled in ice-water. Then the medium was decanted and the slices were transferred into 5 ml of freshly oxygenated HEPES-2; the radioactive morphine was added after temperature equilibration (i.e. after shaking for 30 min at 37°).

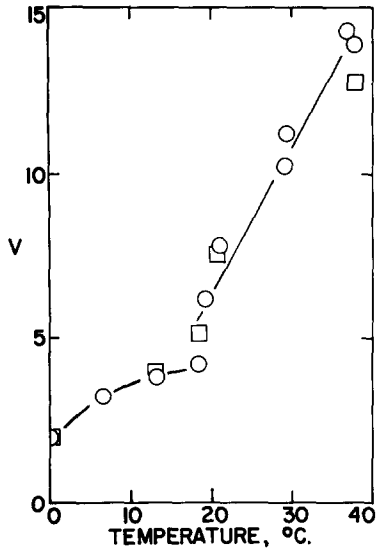
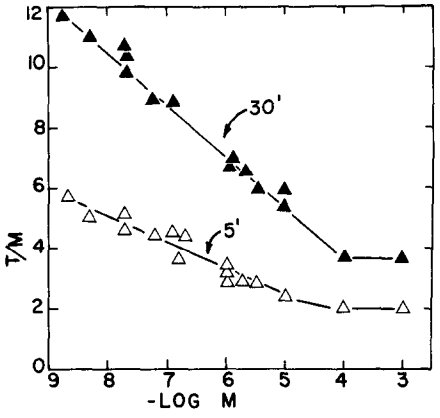


Fig. 4. Rate of morphine uptake into kidney slices is temperature dependent. The media in these experiments contained 31 nM morphine[^{14}C]. Data from 2 to 10 min after the morphine addition (three to four time intervals, three incubations/interval) were used to calculate renal uptake (V) in pmoles/ml of tissue vol/min. Key: rat kidney cortex (□); mouse kidney (○). Estimated standard errors of averages plotted were less than 3 per cent.

In rat kidney slices, uptake was due to renal cortical tissue because rat kidney medulla and collecting tubule regions did not take up morphine (i.e. T/M = 1) at medium levels above 10^{-6} M. Equilibrium in mouse or rat kidney slices was reached after 46 (±4, S. D.) min in 177 control incubations that were stopped at 5-min intervals up to 90 min at 3–1000 nM. Similar results were obtained with guinea pig renal cortex tissue, but the uptake was not as high.

Temperature dependence of uptake. During the linear period after incubation at 31 nM medium levels morphine uptake showed a sharp decrease from 14 pmoles/ml of tissue water/min at 37° to 4–6 pmoles/ml/min at 16–20°. At 0·5° uptake was only 2 pmoles/ml/min for the first 10 min. Similar effects were also observed with slices from rat kidney cortex (Fig. 4). If the slices were suspended in 5 ml medium and heated to 95° in a boiling water bath for 10 min, the uptake of 30 nM or 5 μM morphine was markedly reduced when the slices were transferred into fresh oxygenated medium (Fig. 3).

Saturability of uptake. Measurement of the decrease in T/M with increased M (Fig. 5A) indicated that the morphine uptake might be mediated by at least two mechanisms, one of which was saturable at concentrations less than 10^{-4} M. A plot of M/T vs (−) log M (initial medium concentration) is used to show a



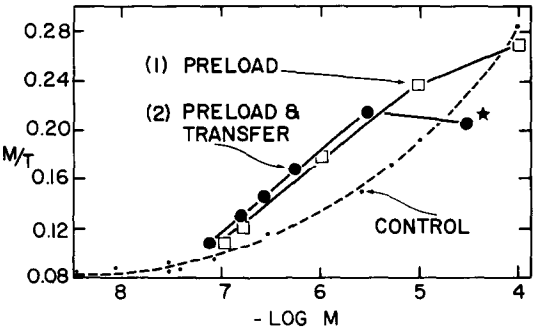


Fig. 5. (A) Morphine uptake by kidney slices is saturable. Both initial (0 to 5-min, △) and equilibrium (30-min, ▲) uptake of morphine decrease with respect to increasing concentrations of morphine in the medium, becoming unsaturable at $>10^{-4}$ M (T/M = 3·7 ± 0·2 S.D.). Data points are averages from 3 to 45 separate incubations at each concentration in HEPES-2. (B) Reciprocal plot of M/T vs M. Prior loading of kidney slices with unlabeled morphine does not increase subsequent uptake of radioactive morphine, nor does the efflux of 'preloaded' morphine. (1) The equilibration medium was loaded with 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} or 10^{-7} M unlabeled morphine, and the slices were incubated for 30 min. Radioactive morphine (80 nM) was added and incubation continued for an additional 30 min. The uptake of the radioactive morphine (□) decreased (curve 1, 'Preload') in parallel with controls represented by the broken line, data taken from (A) above. (2) The equilibration medium was loaded with the same concentrations but the slices were transferred after the first 30 min to fresh medium containing 80 nM morphine[^{14}C] for 30 min additional incubation. Separate experiments (e.g. see Fig. 9) had indicated that 60 per cent of the morphine taken up by slices, from 10^{-3} to 10^{-8} M exited in 30 min after one transfer into fresh medium. Therefore, the abscissa position for the uptake data of the slices transferred from higher morphine concentrations (●, curve 2, 'Preload and Transfer') was placed over the equivalent medium morphine concentration calculated from 80 nM + (60 per cent of the unlabeled tissue morphine/tissue wt/5 ml of fresh medium volume). The concentration of the unlabeled morphine in the tissue was determined from control experiments measuring uptake for the first 30-min equilibration period, with a similar pattern of uptake vs concentration to those data shown in A. The results indicate that this procedure causes inhibition of morphine uptake, with apparent saturation at 5–10 μM. The efflux of large amounts of unlabeled morphine did not increase the uptake of the radioactive morphine, and thus no evidence for counter-transport was observed (with the exception of slices 'preloaded' with 10^{-3} M morphine, data point marked *). The star indicates the only data point that shows evidence for counter-transport, i.e. higher uptake than control or preloaded slices at the same medium concentrations; N = 9, t (independent) = 7·24, P < 0·025. Experimental data points are the averages of three separate incubations with approximately 100 mg of mouse kidney slices.

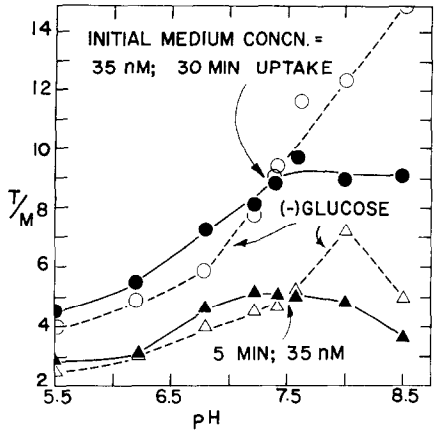


Fig. 6. The pH dependency of morphine uptake in the presence and absence of glucose. Uptake at 5 and 30 min in HEPES-2 medium, containing 10 mM glucose (solid symbols and solid line), and in HEPES-G, containing no glucose (dashed line, open symbols). The pH of the normal HEPES buffer was changed with HCl or NaOH. NaCl added at pH 6·2 did not change the uptake. Estimated standard errors of data points (N = 6) were less than 8 per cent.

distinct change in the type of uptake that occurs at 5×10^{-8} M. A change in M/T vs (−) log M is observed when unlabeled morphine was equilibrated for 30 min with the slices before small concentrations of radioactive morphine were added ('preload') (Fig. 5B). At concentrations above 10^{-4} M, morphine uptake appeared to be unsaturable.

The pH, glucose and ion dependence of uptake. In standard medium (HEPES-2) the uptake was optimal at pH 7·4 to 8 (Fig. 6). In media lacking glucose (HEPES-G) the uptake was enhanced above pH 7·4. At pH 7·4, an increase or decrease of Ca^{2+} or Mg^{2+} had relatively minor effects. The optimal glucose concentration appeared to be 1 mM for uptake, although the absence of glucose in incubations with morphine below 1 μM did not inhibit uptake (Fig. 7). In the presence of glucose, there was an inhibition of morphine uptake by K^+ addition to the medium. However, if the slices were incubated in HEPES-G + (2–10 mM additional K^+) there was up to a 20 per cent increase in the uptake of morphine. At K^+ concentrations above 20 mM the uptake was inhibited (Fig. 8) in HEPES-G.

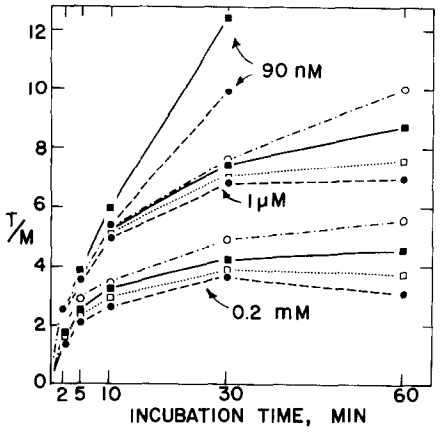


Fig. 7. Effect of varying the glucose concentrations on the uptake of morphine. Each data point represents the average from three separate incubations with 0, 1, 5 or 10 mM glucose in the HEPES medium, represented by (■), (○), (□), and (●) symbols respectively. The sets of data are for uptake of 0·2 mM, 1 μM and 90 nM morphine.

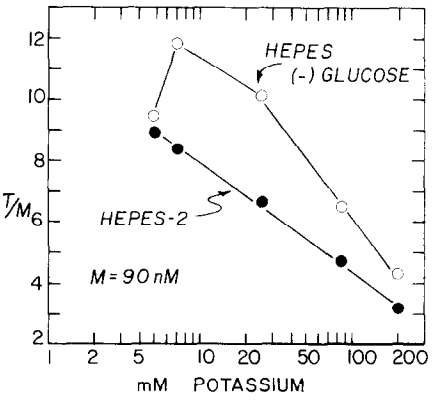


Fig. 8. K^+ can stimulate morphine uptake in the absence of glucose. The morphine concentration was 89 nM and the incubation duration was 15 min at 37°. The potassium was added as KCl and was present throughout the equilibration and incubation period. The normal HEPES-2 medium contains 5 mM K^+. Each point is the average from three incubations.

Oxygen dependence of morphine uptake. If the medium was warmed to 37° and vigorously bubbled with water-saturated N_2 for 10 min and then the slices were equilibrated for 30 min under N_2, the 30-min uptake of morphine was reduced below medium levels from 10^{-8} to 10^{-3} M. (To block uptake, it was not sufficient to just pass N_2 over the surface of the medium, particularly if the latter was still cold from storage.) Tissues treated in this manner never regained any uptake capability if they were transferred to fresh, oxygenated medium after equilibration under N_2. Thus it appeared that even the unsaturable uptake of morphine required viable kidney tissue.

Morphine exit and effects of inhibitors of transport on exit. Although morphine uptake was inhibited by both cyanide and ouabain, at 1 to 5×10^{-4} M both inhibitors could inhibit, and cyanide could also increase exit. If the slices were incubated for short periods in a concentration of CN^- that was mildly inhibitory toward uptake, and the CN^- was washed out during efflux, in some cases the exit of morphine appeared to be retarded or decreased because some of the morphine that did exit was rapidly taken up again by the reactivated tissue. Thus, exit as well as uptake could be inhibited. However, if rapid reuptake of morphine (from unchanged efflux media) was blocked by residual cyanide from the prior incubation step, then it appeared that exit was stimulated.

There was no difference in the time required for 50 per cent of the morphine to exit after 30 min of incubation at 10^{-7}, 10^{-6} or 10^{-4} M. If slower exit in unchanged media was indeed due to reuptake, it did not increase in 10 to 100-fold higher external morphine concentrations, i.e. we did not observe evidence for counter-transport (see also Figs. 9 and 14 below).

Efflux of morphine from the slices was measured in the presence and absence of morphine, antagonists, methadone and other substances. When the incubated slices were transferred into a single efflux solution, the amount of morphine remaining in the tissue was higher after 10–60 min than in slices that were transferred at frequent intervals. This occurred after incubation at 3 to 9×10^{-8} M for 30 min at 37°. However, if the efflux incubation medium contained methadone (3 μM), rotenone or nalorphine (1 μM), the exit of morphine was more rapid. The most rapid exit occurred with sequential transfers or in 1 μM rotenone. The results of these experiments are summarized in Fig. 9. We were unable to determine whether uptake or exit was more sensitive to a metabolic inhibitor, and with these few exceptions most experiments reported here are concerned only with effects upon morphine uptake.

Effects of treatment in vivo *and species differences on morphine uptake.* Slices of rat renal cortex equivalent in mass to those of mouse kidney took up morphine at the same rate from 1 μM initial medium levels, but slices from guinea pig renal cortex took up less morphine at equilibrium (only 40–50 per cent as much as the slices of the other two species) from 30 nM or 2·5 μM medium levels. The equilibrium uptake of 30 nM morphine by renal cortex slices from morphine-tolerant guinea pigs was further reduced 30 per cent from untreated controls. Such reduced uptake was not affected by 10 μM methadone, levorphanol or nalorphine but was inhibited 75 per cent by 1·5 μM rotenone, as in slices from untreated animals. Morphine uptake by kidney slices from rats made tolerant to or withdrawn from morphine did not show significant changes from controls.

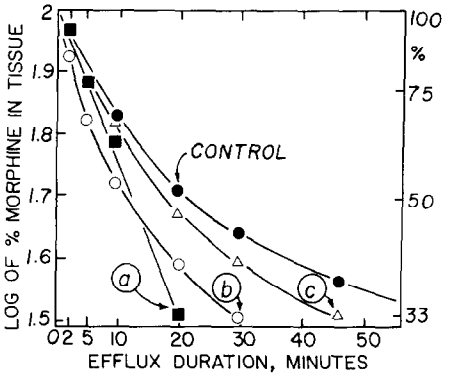


Fig. 9. Effects of morphine, methadone, nalorphine, and rotenone on the efflux of morphine from kidney slices, semilogarithmic plot. After 30 min of incubation in 35 nM morphine[^{14}C], the slices were transferred into fresh HEPES-2 containing one of the following: (a) 3·5 or 8·0 μM morphine, or 1 μM rotenone, or 1 μM nalorphine (■); (b) 3 μM methadone (○); or (c) 88 nM morphine (△). Efflux without additional drug in the exit medium is shown by the line marked 'control' (●). If the slices were transferred to fresh media at each time point, the efflux followed the same pattern as that in rotenone (a) (■). The left-hand ordinate, marked in even intervals, is the log of the percentage of morphine[^{14}C] remaining in the slices; the right-hand ordinate is the percentage of the morphine[^{14}C] remaining in the tissue. Data points are averages of six to nine incubations with estimated standard errors less than 6 per cent.

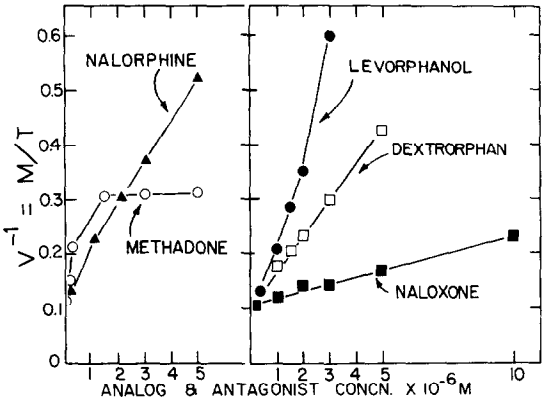


Fig. 10. Inhibition of morphine uptake by narcotic antagonists and analogs. Test compounds were equilibrated with the slices 30 min before 30 nM [^{14}C]morphine was added for a 20-min incubation. The ordinate scale, v^{-1} = (morphine in medium/morphine in tissue volume) = M/T. In contrast with these results, no inhibition of 10^{-6} M morphine uptake was observed with 5×10^{-6} M levels of the antagonists or analogs. Data points are averages from six to twelve separate incubations with estimated standard errors less than 7 per cent.

Inhibition of morphine uptake in kidney slices

Effects of narcotic analogs, antagonists and other bases or alkaloids on morphine uptake into kidney slices. Levorphanol, nalorphine, dextrorphan, methadone or naloxone was equilibrated with slices prior to addition of [^{14}C]morphine (Fig. 10). Below 1 μM, methadone was most effective in inhibiting the uptake of morphine, with levorphanol and nalorphine next most inhibitory, followed by dextrorphan, while naloxone was least effective at any concentration. Methadone inhibition was not complete or proportional to concentration and essentially ceased at

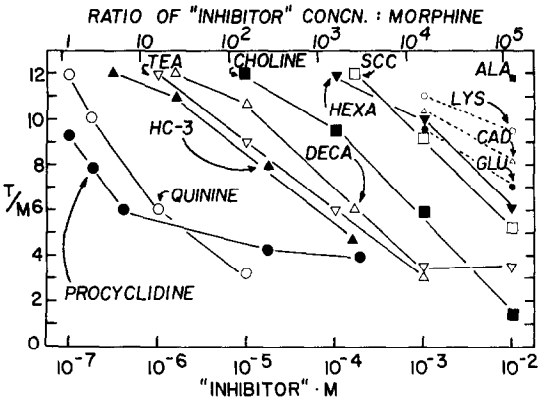


Fig. 11. Inhibition of 80 nM morphine uptake into mouse kidney slices by some quaternary ammonium salts, amino acids and bases. Nonstandard abbreviations are: TEA, tetraethylammonium; HC-3, hemicholinium; DECA, decamethonium; HEXA, hexamethonium; SCC, succinylcholine; and CAD, cadaverine. All 'inhibitors' were equilibrated with the slices at 37° for 30 min before [^{14}C]morphine was added for a 30-min incubation. Data points are averages from three incubations. Control incubations with addition of H_2SO_4 at the concentrations used with quinine, i.e. up to 50 μl of 0·1 N H_2SO_4, showed no effect on uptake.

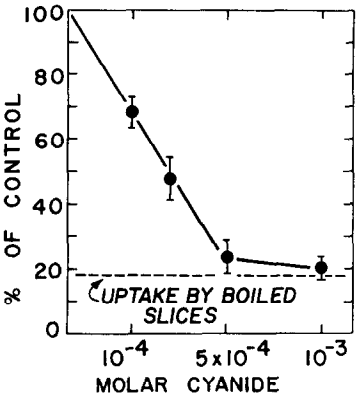


Fig. 14. Inhibition of 5- and 30-min uptake of morphine from 80 nM medium levels by cyanide. In these experiments, cyanide and morphine were added simultaneously. The bars represent the range of the standard deviation of the averages (●) from twelve incubations. There was no significant difference between the effect of CN at 5 or at 30 min on the morphine uptake; 50 per cent inhibition of uptake was produced by $2{\cdot}5 \times 10^{-4}$ M CN, but uptake was not quite reduced to the level obtained with boiled slice controls (------) even at 0·5 to 1 mM CN.

acid transport into kidney slices was also blocked by rotenone to the same extent as morphine uptake (Table 4). Figure 16 shows the effect of antimycin-A and oligomycin on morphine uptake in the presence and absence of glucose. Whether glucose was present or not, or was replaced with succinate (cf. Discussion), the inhibition by antimycin-A remained the same, but inhibition by oligomycin was reduced when glucose

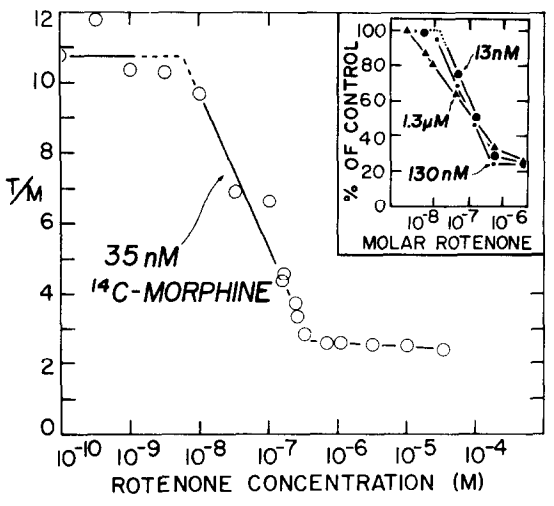


Fig. 15. Inhibition of 30-min morphine uptake into mouse kidney slices by rotenone. Conditions are same as in Figs. 11 and 13; the inset shows that similar results were obtained with rotenone on the equilibrium uptake of morphine using 13 nM, 130 nM and 1·3 μM morphine levels in the medium: 10^{-7} M rotenone caused 50 per cent inhibition of morphine uptake. Data points are averages from three incubations at each rotenone concentration. The rotenone solutions were prepared from clear, cold 100 mM stock concentrates in acetone, by serial dilution. The final rotenone solution added to each incubation flask was 75% (v/v) acetone in water. Acetone concentrations were kept below 10^{-3} M in the medium. The room was darkened for all of these incubations to prevent photodecomposition of the rotenone.

Table 4. Effect of rotenone on morphine and D-glutamate uptake by kidney and brain slices

	Rotenone (μM)	T/M after 30 min*	% of control
Kidney slices			
D-Glutamate (1 mM)	0	2·46	100
	1·5	0·62	25·3
Morphine (31 nM)	0	8·94	100
	1·5	1·99	22·3
Brain slices			
D-Glutamate (1 mM)	0	19·9	100
	1·5	4·21	21
Morphine (31 nM)	0	2·6	100
	1·5	2·5	96

* Slices were equilibrated with rotenone for 30 min at 37° before the radioactive D-glutamate or morphine was added. Values are averages from six to fifteen incubations with estimated S.E.M. less than 6 per cent.

was replaced by succinate. A maximal inhibition obtainable with oligomycin was 64 per cent under the latter conditions. Valinomycin, an inhibitor of K^+ transport, was also examined in a similar set of experiments, but no difference was obtained if glucose was replaced in the medium by succinate (Fig. 16). Inhibition by nonyl-hydroxyquinoline *n*-oxide was similar to that of antimycin-A.

In contrast to these strong inhibitors, other uncoupling agents, e.g. amytal, quinacrine and chloramphenicol, had a lesser effect. 2,4-DNP (see Fig. 12) caused up to 20 per cent less inhibition of the 5-min morphine uptake than it did of the equilibrium uptake at 30 min. Previously, Piccoli and Lajtha [13] showed that 3 μM of 2,4-DNP reaches equilibrium in 30 min in brain and it may require longer equilibration with such compounds to obtain constant inhi-

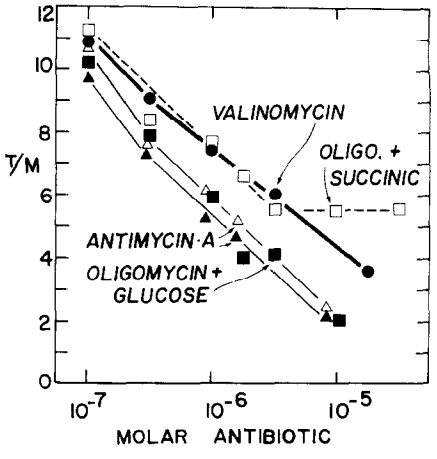


Fig. 16. Extent of inhibition of 350 nM morphine by oligomycin depends upon the presence of glucose or succinate, while that due to antimycin-A and valinomycin does not. Symbols used are: solid, in HEPES-2 with glucose; open, in HEPES (−) glucose, + succinate: triangles, antimycin-A; squares, oligomycin; and circles valinomycin. If glucose was absent, it required a 3-fold increase in the concentration of oligomycin to produce the same inhibition as when glucose was present. Data points are averages from three incubations.

Table 5. Effects of inhibitors with Krebs' cycle substitutions for glucose in HEPES-G medium: T/M at 30 min

Additions	Succinate (1 mM)	Lactate (1 mM)	α-Ketoglutarate (1 mM)	Glucose (1 mM)
None	9·96 = 100%	78·2%	82·8%	134%
PMS (2·5 μM)	29·1*	57·9	23·3	50·1
PMS (2·5 μM) + ascorbate (32 μM)	53·3	33·6	34·2	46·3
PMS (2·5 μM) + ascorbate (32 μM) + Rotenone (1·5 μM)	36·1	68·5	38·4	39·2
Rotenone (1·5 μM)	22·2	26·2		27·1
Malonate (50 mM)	10·3	16·5	12·5	15·4
Malonate (10 mM)	25·5	60·1	30·4	30·5
Malonate (10 mM) + succinate (10 mM)	60·2	71·1	74·5	75·2
Chlorpromazine (10 μM) (or, +PMS; or +ascorbate)	31·2			35·4

* Each value is the average of three incubations using mouse brain slices and 80 nM morphine-[^{14}C], as per cent of control, 9·96 T/M. Similar results were obtained with 30 nM and 8 μM morphine.

bition of initial and equilibrium uptake. Chloramphenicol (10^{-6}–10^{-4} M) had no effect on uptake, while quinacrine at 80 μM inhibited uptake 58 per cent (higher quinacrine concentrations interfered with counting procedures). Inhibition of 31 nM or 1·3 μM morphine uptake increased linearly from 15 per cent at 10^{-5} M amytal to 45 per cent at 2 mM. No effect of gramicidin-D was observed that was not also produced by the acetic acid solvent.

Electron transfer traps and Krebs' cycle substitution. Relatively mild reducing agents, such as ascorbate or methylene blue, inhibited morphine uptake. Results of the addition of various electron acceptor and substrate substitutions are summarized in Table 5. Phenazine methosulfate alone, or in combination with ascorbate, inhibited morphine uptake strongly. Part of the PMS inhibition with succinate was relieved by ascorbate, but the reducing agent increased PMS inhibition with lactate. Partial reversal of rotenone inhibition also occurred when PMS and ascorbate were added to all substrates, being most notable with lactate. Malonate inhibited uptake the least with lactate, and its inhibition was partially reversed by additions of succinate to all other substrates. Inhibition by CPZ (using succinate or glucose) was not ameliorated by PMS or ascorbate.

DISCUSSION

Mode of morphine entry into kidney slices. Morphine appears to enter the kidney by at least three independent mechanisms. First, we obtained evidence for entry by diffusion, shown by uptake in boiled tissue with T/M > 1, although transport mechanisms were inactivated. Second, concentrative mechanisms, requiring metabolic energy and inhibited by specific mitochondrial enzyme poisons, as well as general metabolic inhibitors, appeared to derive energy from Krebs' cycle intermediates substituted for glucose. The uptake of morphine from 5×10^{-7} to 5×10^{-5} M appeared to satisfy criteria for active transport processes via concentrative, energy-dependent mechanisms. Third, unsaturable uptake was observed in loading experiments at concentrations above 10^{-4} M. The tissue/medium levels under these conditions were relatively constant at 3 to 4-fold the external medium concentrations. This type of morphine uptake occurs with brain slices [8], with other drugs in both brain and kidney [6, 7], and at morphine concentrations greater than 10^{-5} M with liver slices (unpublished results). Hug [5] also showed that this occurs with dog kidney uptake of dihydromorphine.

Indirect evidence for a fourth type of morphine uptake, binding (inherent to carrier-mediated concentrative mechanisms described above), was obtained in experiments with morphine concentrations lower than 50 nM in which analogs inhibited uptake and prevented reuptake. However, when the medium morphine concentrations were more than 1 μM, the inhibition by the analogs was no longer observed. If all of the morphine uptake was due to binding, we could have expected to observe evidence for some stoichiometry between the tissue mass and amount of morphine removed from the medium, with a T/M decreasing sharply to a constant value within at most two orders of magnitude change in medium concentration. Even with a weak affinity of morphine for the tissue, when the medium concentration was increased 100-fold, binding sites would have been relatively saturated and additional uptake would have stopped, unless the morphine in the tissue was removed to another compartment by a carrier or conversion to a metabolite. Moreover, the efflux time for the exit of 50 per cent of the tissue load of morphine would have varied inversely with the initial medium concentration if the morphine were bound internally in the slice. Instead, the efflux time was uniform after varous loads. We did not observe any evidence for morphine metabolism, and the T/M did not decrease so sharply with increments in M that we could determine what the number of binding sites were per g of tissue. Therefore, although both binding and transport processes are saturable, and binding is a first step in carrier-mediated transport, the degree of saturability is greater when only binding is involved, and

we observed no evidence other than limited structural analog competition to suggest that morphine uptake in kidney involved binding alone.

Type of inhibition of uptake. Our results with morphine generally are in accord with what had been previously reported by Bell [4], Hug *et al.* [1] and Hug [5] for dihydromorphine in kidney. Because the characteristics of morphine uptake into brain slices appeared to be different from those reported for dihydromorphine [8], we decided to reinvestigate morphine uptake in kidney slices, particularly from mice, using various inhibitors of transport and enzyme activity. Some of the inhibitory compounds had been shown to interfere effectively with dihydromorphine uptake into dog kidney slices, e.g. IAc, 2,4-DNP, FAA and NaCN [1], nalorphine, probenecid, levorphanol and dextrorphan [5]; or into choroid plexus, e.g. decamethonium and hexamethonium [5]. Some potentially inhibitory compounds were shown to be accumulated in rat and chicken kidney slices (hexamethonium and tetraethylammonium) during investigations of the binding of organic bases [14]. Many of the others had been used in our laboratory to study energy requirements for amino acid transport in brain slices [10, 11].

The presentation of drug uptake data in pseudo-Lineweaver–Burk format is not entirely valid for morphine uptake by kidney slices for the following reasons. First, the choice of a baseline would be arbitrary since boiled tissue, tissue incubated under N_2 atmosphere, or CN-treated tissue each showed a different reduction of morphine uptake. Although boiling the slices undoubtedly destroyed membranes and reduced the equilibrium uptake to a T/M of <3, incubation under N_2, which might be considered a milder treatment, caused much greater inhibition to a T/M of 0·8. Second, the uptake of morphine was not due to passive binding. Thus the effects of inhibitors of such uptake might appear to be noncompetitive when examined with standard graphical analyses (i.e. reciprocal plotting of T/M vs I), but the inhibitor could be acting on an entirely different component of the uptake process, e.g. depleting the available energy required to concentrate the morphine within the tissue. At present we have too little information concerning the degree of coupling of energy-producing and carrier systems to simply reduce them to a single component for graphical analysis. Third, some inhibitors (e.g. 2,4-DNP) had little effect on the initial rate of morphine uptake but decreased the equilibrium levels.

Factors controlling morphine uptake: competition for carrier by structural analogs and other organic bases. The concentrative uptake was sensitive to potent mitochondrial enzyme poisons such as rotenone or antimycin-A. However, the bulk transport (from medium levels above 10^{-6} M) of morphine did not show the same sensitivity to inhibition by analogs or pharmacological antagonists as did morphine uptake from medium concentrations below 10^{-7} M. Methadone may be a stronger inhibitor of morphine uptake than levorphanol or nalorphine at relatively low concentrations due to the extremely high affinity of the tissue for methadone [15]. Furthermore, although levorphanol and nalorphine appeared to be more potent than dextrorphan in blocking morphine uptake, the relative lack of inhibition of morphine uptake by naloxone indicates that, for the kidney slice experiments, we were probably not observing stereospecific, receptor-type binding.

Differences in inhibition of morphine uptake by various organic bases may be due, in part, to their accumulation to different levels in kidney slices. McIsaac [14], for example, showed that 10 μM TEA was accumulated by rat kidney 4- to 5-fold above the medium, while 10 μM decamethonium was accumulated only 1·5 to 2·5-fold. In accord with his observation, we observed that TEA was about twice as effective as decamethonium in inhibiting morphine uptake by mouse kidney. The same compound may also be more inhibitory toward one type of organic base than another. Whereas McIsaac demonstrated that quinine sulfate at 0·2 mM inhibited 10^{-5} M TEA uptake by 50 per cent, in these experiments the morphine uptake was twice as sensitive: 0·8 μM quinine inhibited 80 nM morphine uptake by 50 per cent.* Furthermore, some differences between our findings and those reported by previous investigators who measured drug effects on renal morphine uptake may be due to a peculiarity of the sensitivity of the tissue from a particular species. For example, we observed decreased uptake in guinea pig kidney slices in comparison with tissue from mice or rats.

Of the other compounds examined, choline is taken up to high levels by kidney tissue yet appears to be a very weak inhibitor of morphine uptake in comparison with hemicholinium or TEA. This suggests that the renal morphine uptake may be poorly controlled by the intracellular organic base concentrations. In addition, loading of slices with up to 50 μM nalorphine, levorphanol, dextrorphan or naloxone had no effect on 10 μM morphine uptake, despite the fact that nalorphine, levorphanol annd dextrorphan are all accumulated much above medium levels of 10^{-5} M by kidney tissue [5]. Indeed, Hug has shown that mM nalorphine is required to inhibit $\frac{1}{3}$ of the dihydromorphine uptake from 10 μM medium levels in dog kidney slices, an 'inhibitor substrate' index of 100 for 33 per cent inhibition. In the present study, a 100-fold higher concentration of nalorphine inhibited uptake of 30 nM morphine by 75–80 per cent. This apparent sensitivity of the morphine uptake by kidney slices at low concentrations to inhibition by analogs and antagonists (at low concentrations) is suggestive of a competition for binding sites but is not necessarily indicative of inhibition of the transport process for the following reasons.

First, for binding precedent to transport, inhibition diminishes as the absolute morphine concentration in the medium increases, provided that the effect of such competitive inhibition is primarily upon a first step in the total uptake process. This was observed in these experiments. Second, however, binding may also occur within the tissue to terminate uptake, as shown by Holm [16] for decamethonium. If the internal binding of the first (analog or antagonist) compound is at a site where the morphine must finally bind, inhibition of uptake would be expected to increase

*The common admixture of quinine with heroin may block the excretion of morphine *in vivo* and amplify the effects of the illicit drug.

Table 6. Comparison of characteristics of morphine and amino acid uptake in mouse or rat brain and kidney

	Brain		Kidney	
	Amino acid uptake	Morphine uptake	Amino acid uptake	Morphine uptake
Saturable	+	−	+	+
Temperature sensitive	+	±	+	+
High-affinity components	+	+	+	+
Low-affinity components	+	+	+	+
Inhibited strongly by:				
Anoxia	+	−	+	+
μM mitochondrial poisons	+	−	+	+
mM general poisons	+	−	+	+
Depletion of glucose	+	−	−	−
mM solvents	−	−	+	+
mM SH-oxidants	+	−	+	+
pH change	+	±	+	+
mM ouabain	+	−	−	−

exponentially as the concentration of the analog or inhibitor increases. Such was the case with relatively low concentrations of levorphanol (below 3 μM) but did not occur consistently with the other analogs or antagonists. Third, furthermore, the finding of a uniform exit time for efflux of various loads of morphine does not support the hypothesis of internal binding sites.

In this study we did not attempt to increase the concentration of morphine analogs and antagonists above 50 μM because above this concentration they begin to interfere with tissue respiration [4], and other functions in a nonspecific manner [17]. Moreover, the increase in efflux of morphine after addition of CN, rotenone, methadone or nalorphine did not indicate any structural specificity to suggest that the narcotic or antagonist was competing with morphine for some internal binding site. Thus, despite some apparent stereospecificity among the analogs inhibiting 10^{-8} M morphine uptake, it appears that morphine is not bound in the kidney slice by passive effects (i.e. an affinity of association with binding sites in competition with structural analogs) but that active metabolic processes retain an internal, diffusible pool of the drug.

Role of mitochondrial metabolism in renal morphine uptake. Morphine uptake is metabolically sensitive, i.e. if the kidney tissue was heated to 95° for 10 min or poisoned with strong inhibitors of mitochondrial function, the slices no longer took up morphine, even from 10^{-8} M medium levels. This is quite different from the relative insensitivity of morphine uptake by brain slices [8]. In fact, the lowest T/M values (80 per cent inhibition) were obtained with three treatments that block mitochondrial energy production: complete anoxia (under N_2), in the presence of more than 1 μM rotenone, 1 mM cyanide or 50 mM malonate, regardless of the substrate replacing glucose as a source of energy for uptake. An inhibitory effect of glucose on morphine uptake was observed in this study, but inhibitors of glucose transport or of glycolysis had little or no effect on morphine uptake, although they stimulate amino acid uptake by rat renal cortex [18,19]. Because some of the glucose-transport inhibitors, as well as atractyloside, were inactive, we cannot show positive evidence of their penetration into the tissue. Therefore, we cannot directly demonstrate the glucose requirements for morphine transport into kidney slices.

We were able to maintain uptake of morphine from 30 nm to 10 μM medium levels with various Krebs' cycle substrates: succinate and α-ketoglutarate being most efficient. Inhibition caused by malonate was reversible by succinate, while that due to rotenone was partially reversed with PMS and ascorbate. These effects suggest that transport of morphine requires mitochondrial, rather than glycolytic, activity. The reduction of inhibiton by oligomycin when succinate was substituted for glucose also suggests the possibility that phosphorylated intermediates other than ATP may be used for active transport. The inhibition of uptake by K^+ ion and the relative resistance of the morphine uptake to ouabain (in comparison to brain amino acid transport) suggest that ion flux may not be a driving force for renal morphine transport.

Comparison of morphine uptake by kidney slices and amino acid uptake by brain slices. Comparison of the results of these experiments with those using brain slices [8] indicates that morphine uptake in kidney is very different from that in brain but has many characteristics that are similar to those of amino acid transport in brain (Table 6). However, the kidney slice uptake of morphine is much more sensitive to inhibiton by alcohol, acetone or K^+ and is much less sensitive to NaF, CPZ and ouabain than is the brain slice uptake of amino acids, while the mass of amino acids that can be taken up by brain slices is much greater than that of morphine, amino acids or TEA into kidney tissue (i.e. the capacity at equilibrium for valine or leucine in brain is more than 5–10 mM from 2 mM medium). Despite these differences, certain aspects of mitochondrial function appear to be required for transport in both tissues.

Acknowledgement—Portions of this work were supported by U.S. Public Health Service Grant R-5707.

REFERENCES

1. C. C. Hug, Jr., L. B. Mellett and E. J. Cafruny, *J. Pharmac. exp. Ther.* **150**, 259 (1965).

2. W. P. Baker and L. A. Woods, *J. Pharmac. exp. Ther.* **120**, 371 (1975).
3. J. F. Fujimoto, in *Narcotic Drugs. Biochemical Pharmacology* (Ed. D. H. Clouet) p. 366. Plenum Press, New York (1971).
4. J. L. Bell, *J. Neurochem.* **2**, 265 (1958).
5. C. C. Hug, Jr., *Biochem. Pharmac.* **16**, 345 (1967).
6. D. N. Teller, T. DeGuzman and A. Lajtha, *Trans. Am. Soc. Neurochem.* **3**, 128 (1972).
7. D. N. Teller, T. DeGuzman and A. Lajtha, *Psychopharmacologia* **26** (CINP suppl.), 120 (1972).
8. D. N. Teller, T. DeGuzman and A. Lajtha, *Brain Res.* **77**, 121 (1974).
9. D. N. Teller, T. DeGuzman and A. Lajtha, *Trans. Am. Soc. Neurochem.* **4**, 139 (1973).
10. M. Banay-Schwartz, D. N. Teller and A. Lajtha, *Trans. Am. Soc. Neurochem.* **5**, 93 (1974).
11. M. Banay-Schwartz, D. N. Teller, A. Gergely and A. Lajtha, *Brain Res.* **71**, 117 (1974).
12. A. L. Misra and S. J. Mulé, *Biochem. Pharmac.* **21**, 193 (1972).
13. F. Piccoli and A. Lajtha, *Biochim. biophys. Acta* **225**, 356 (1971).
14. R. J. McIsaac, *J. Pharmac. exp. Ther.* **168**, 6 (1969).
15. D. N. Teller, T. DeGuzman and A. Lajtha, *Trans. Am. Soc. Neurochem.* **5**, 180 (1974).
16. J. Holm, *Biochem. Pharmac.* **22**, 983 (1973).
17. J. M. Hiller and E. J. Simon, *J. Neurochem.* **20**, 1789 (1973).
18. S. Segal, S. Thier, M. Fox and L. Rosenberg, *Biochim. biophys. Acta* **65**, 567 (1962).
19. S. Segal, A. Blair and L. E. Rosenberg, *Biochim. biophys. Acta* **71**, 676 (1963).