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Uptake of [7, 8-^{3}H]dihydromorphine by rat cerebral cortical slices and eye tissue

Morphine and its congeners have recently been reported by Scrafani & Hug, (1968) to be accumulated in rat cerebral cortical slices by an active transport process. We now report an inability to saturate the uptake system at concentrations ranging from 2·5 to 20·0 μg/ml likely to be encountered *in vivo* and the inability of glucose-substrate or metabolic inhibitors to depress the uptake of [7,8-^{3}H]dihydromorphine.

Male Holtzman rats, 140–200 g, were decapitated, the brains placed in a cold chamber and tissues sliced by an apparatus (O'Neill, Simon & Cummins, 1963) adjusted to give two outer slices, one dorsal and one lateral (weight, 15–25 mg, thickness, 0·2 mm) from each hemisphere. The slices were transferred to incubating beakers (20 ml) containing 2 ml oxygenated calcium-free Ringer (Elliot, Kokka & Way, 1963), previously kept in ice, and dihydromorphine, and incubated in Dubnoff metabolic shaker at 37° under oxygen with a shaking rate of 140 strokes/min. Control samples prepared as above were kept at 0° without shaking. At the end of the incubation, beakers were quickly placed on ice, tissue slices rinsed several times with saline, transferred to tared aluminium foil and dried to a constant weight at 105° and their radioactivity assayed.

The accumulation of dihydromorphine (2·2 μCi/mg) by slices in oxygen at 37° reached a steady-state distribution between tissue and medium in about 30 min (Fig. 1a). The uptake of dihydromorphine by slices was linear and unsaturable at concentrations of 2·5 to 20 μg/ml (Fig. 1b). The tissue/medium ratio (T/M) showed slight changes as dihydromorphine concentration was increased in the incubating solution. These findings were not altered with the omission of glucose from the media. At low concentrations of dihydromorphine (1·25 μg/ml) the T/M ratio was greater than at other concentrations studied. Neither the addition of calcium ($1{\cdot}3 \times 10^{-3}$M) to incubating solution nor 3-day fasting of animals changed the effect of glucose on the uptake of dihydromorphine. Glucose had no effect on accumulation of dihydromorphine in tissue slices when the concentration of dihydromorphine was 1·25 μg/ml. Increasing glucose concentrations from 0 to 12 mM at 37° produced a corresponding decrease in dihydromorphine uptake, which could not be accounted for on the basis of slight pH changes alone during incubation for 30 min.

Dinitrophenol, nitrogen atmosphere and high potassium content in the medium (an additional 1×10^{-1}M) significantly inhibited the accumulation of dihydromorphine in the slices (Table 1). Sodium cyanide, sodium malonate, iodoacetate, fluoroacetate had no effect on accumulation of dihydromorphine in slices with or without glucose in incubating medium.

Although nalorphine at a concentration of 2·5 μg/ml and with a 15 min preincubation period before the addition of dihydromorphine to the medium, did not alter the accumulation of dihydromorphine in the tissue, pretreatment of rats with a subcutaneous injection of nalorphine, 20 mg/kg, 30 min before decapitation, produced a significant decrease in tissue accumulation of dihydromorphine when the incubating solution contained no glucose but there was no effect when glucose was in the medium.

Our failure to inhibit [^{3}H]dihydromorphine transport by omission of glucose from incubation media, or by known metabolic inhibitors and also the failure to saturate this transport system in the concentration range 2·5–20·0 μg/ml of [^{3}H]dihydromorphine furnish evidence that an active transport mechanism is not involved. These results are at variance with those of Scrafani & Hug (1968), but agree with those of Bell (1958) and Miller & Elliott (1954).

For the measurement of uptake of [^{3}H]dihydromorphine in rat eye tissue, the rats were injected subcutaneously with a dose of 10 mg/kg of the drug and after the required time, decapitated, the eyes removed, freed from adhering tissue, washed with physio-

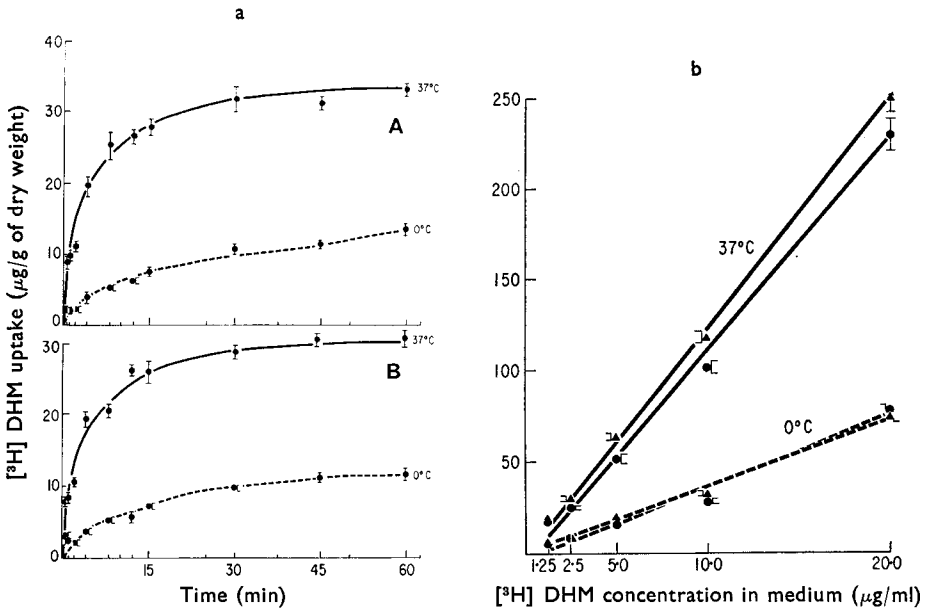

FIG. 1a. Rate of uptake of [^{3}H]dihydromorphine by rat brain cortical slices. Dihydromorphine concentration in medium 2·5 μg/ml, pH 7·4 in (A) absence and (B) presence of 12 mM glucose. Each point represents the mean of 7 or 8 samples. b. Relation of concentration of [^{3}H]dihydromorphine to its uptake by rat brain cortical slices. Incubation time is 30 min, pH 7·4. Each point represents the mean of 7 or 8 samples.

Table 1. *Effect of various agents upon the uptake of* [^{3}H]*dihydromorphine* (2·5 μg/ml) *by rat brain cortical slices after* 30 *min incubation at* 37° *at pH* 7·4

Agents	Concentration	% of controls* (± s.e.)	$P \leq$
Dinitrophenol	1×10^{-3}	74·3 ± 2·3	0·001
Nitrogen atmosphere	—	88·9 ± 2·3	0·01
Nitrogen atmosphere (without glucose)	—	79·8 ± 1·2	0·001
Nalorphine (*in vivo*)	20 mg/kg s.c.	85·9 ± 4·4	0·02
Additional potassium chloride	1×10^{-1}	90·6 ± 4·0	NS
Additional potassium chloride (without glucose)	1×10^{-1}	78·5 ± 3·8	0·001
Sodium cyanide	1×10^{-3}	95·5 ± 4·4	NS

* Mean of 8 samples. NS = not significant.

Iodoacetic acid (1×10^{-3}M), malonic acid (1×10^{-3}M), sodium fluoride (1×10^{-3}M), sodium fluoroacetate ($3·3 \times 10^{-3}$M), calcium chloride ($1·3 \times 10^{-3}$M) and nalorphine (2·5 μg/ml) did not alter the uptake.

logical saline, gently wiped with tissue paper and, after weighing, transferred to counting vials. Eyes and slices moistened with 2–3 drops of water were dissolved in 0·5–1·0 ml NCS solution (a quaternary ammonium base supplied as a 0·6N solution in toluene by Amersham/Searle Corp., Des Plaines, Illinois) by gentle warming and shaking at 45°. After dissolution of the tissue, 10 ml of phosphor-toluene was added, the contents were well mixed, and the radioactivity assayed in a liquid scintillation counter. The values were corrected for quenching by the addition of 1 ml of internal standard [^{3}H]toluene. Aliquots of incubating fluid were similarly assayed. The uptake of [^{3}H]dihydromorphine (10 mg/kg s.c.) in two eyes for each animal each at different time intervals after injection was (ng/g of tissue): 980, 1451, 1059, 437, 245, 168 at times after injection of 30, 60, 120, 240, 360 and 720 min respectively. The values obtained give no indication of the amount of drug actually present in the lens. The peak value of drug was reached approximately 60 min after injection.

Thin-layer chromatography of organic extracts of homogenized eyes and cortical tissue slices with solvent system ethyl acetate–methanol–conc. ammonia (17 : 2 : 1 v/v) which can resolve oxidation and dealkylation products of dihydromorphine, and paper chromatography with n-butanol–conc. ammonia and water (4 : 1 : 3 v/v), did not give evidence of metabolic conversion of dihydromorphine. A single peak of radioactivity due to dihydromorphine was observed in both cases.

The general pattern of uptake of dihydromorphine *in vivo* in eye tissue is similar to that for brain and plasma levels (Sanner & Woods, 1965; Hug & Mellett, 1962). The presence of a high concentration of reduced glutathione and its active synthesis has been demonstrated in mammalian lens by several workers and it has been shown (Kinsey & Merriam, 1950; Salmony, 1960; Sippel, 1966) that the content of this tripeptide in experimental or senile cataract is drastically diminished. Evidence for interaction of morphine and reduced glutathione to form a peptide-conjugate has recently been obtained (Misra & Woods, 1970). In view of the absence of bio-transformation products of dihydromorphine in eye tissue, the formation of reversible lenticular opacity by morphine-like analgesics may conceivably be due to some interference with lens metabolism or inhibition of glutathione synthesis.

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