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DISTRIBUTION OF TRITIUM-LABELLED ETORPHINE (M99) AND DIHYDROMORPHINE IN PREGNANT RATS AT TERM

BY

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A recent report from this laboratory showed indirectly that maternally administered etorphine readily crosses the placenta of the rat into the circulation of the foetus (Blane, 1966). Etorphine is one of the most potent analgesics described to date, and in adult animals causes many of the side-effects which are typical of morphine-like agents (Blane, Boura, Fitzgerald & Lister, 1967). When given to pregnant rats there is respiratory depression in the mother, and the young are born with oxygen uptake depressed in direct proportion to the maternal dose. Low doses of etorphine resulted in less neonatal depression than did equianalgesic doses of morphine or methadone, although in very large doses it caused a proportion of neonatal deaths soon after delivery. Morphine was not toxic in this respect and methadone only marginally so.

The investigation described here was undertaken to obtain information on the distribution of labelled etorphine between maternal and foetal tissues after administration of the drug to female rats at term. The tritiation of etorphine is relatively straightforward. Tritiated morphine would have been the reference standard of choice, however, it is impracticable to label morphine in a specific non-labile position and recourse was therefore made to the use of dihydromorphine, which may be prepared from morphine by reduction with tritium gas. Substitution of dihydromorphine for morphine is permissible, since the pharmacological profile of the two drugs is almost identical, with dihydromorphine slightly less potent than the parent alkaloid in all tests (for references see Hug & Mellett, 1963). The distribution and metabolism of the two drugs is also very similar, if not identical (Hug & Mellett, 1965).

METHODS

Preparation of radioactive drugs

Etorphine [7-(1-(*R*)-hydroxyl-1-methylbutyl)-6,14-endoetheno tetrahydro-oripavine], formerly known as M99(Reckitt) was labelled in the non-labile 8-position by Dr. A. McCoubrey and details of the method have been published separately (Lane, McCoubrey & Peaker, 1966). In brief, tritiated formaldehyde was used to incorporate the required label at an early stage in the synthesis of etorphine from thebaine. The sample was repurified by thin-layer chromatography on silica and development with ether immediately before use. The spot corresponding to the pure product was eluted and the elution solvent evaporated. The residue was dissolved in saline, converted to the hydrochloride, and the concentration in 0.9% saline solution adjusted to 12 μg/ml. The specific activity of the purified etorphine was 48 mc/m-mole.

Tritium-labelled dihydromorphine was prepared by catalytic reduction of the 7,8-double bond of morphine. The purified product was diluted with non-radioactive dihydromorphine and converted to the hydrochloride in saline to give a 27 mg/ml. solution. The specific activity of the diluted dihydromorphine was 0.145 mc/m-mole. Radiochemical purity of this material was checked by thin-layer chromatography.

Administration of drugs to the mothers

The tritiated drugs in saline solution were injected at a predetermined dose level into the left gastrocnemius muscle of 21-day pregnant rats. A S.P.F.-derived Sprague Dawley strain of rat having a mean body weight at term of 310 g was used. The doses of the respective hydrochlorides were 12 μg/kg etorphine and 27 mg/kg dihydromorphine, which are roughly equipotent as analgesics and represent approximately five times the normal ED80. The choice of this rather high dose-level was conditioned mainly by the difficulty in estimating accurately very low concentrations of tritiated etorphine in tissues.

Collection and assay of tissues

Tissue levels were determined at 10, 15, 20, 60 and 120 min after drug administration to the mothers and at least six rats were sacrificed at each point in time. At the appropriate interval after injection a sample of the maternal blood was taken by cardiac puncture and transferred to a wax impregnated rice paper cachet. Subsequently the mother was immediately killed by dislocation of the neck and the foetuses delivered by Caesarean section. A single foetus was placed in a cachet and killed. The remaining foetuses were decapitated and the blood issuing from their trunks collected with a heparinized Pasteur pipette and pooled. The brains from the decapitated foetuses were expelled through the base of the skull by squeezing the head with forceps and collected in a single cachet. Three placentae cleaned of mucus and extraneous blood were also pooled in a single cachet. The final dissection entailed removal of the maternal brain. Clean instruments were used for the removal of each tissue to avoid cross contamination. The collected tissues were retained in their rice paper cachets, weighed, and dried over phosphorous pentoxide under vacuum.

Each cachet containing dried tissue was tied to the platinum platform of an oxygen flask combustion apparatus (Dobbs, 1966). The flasks were evacuated and filled with oxygen. The sample was ignited by an electrical discharge, and the flask allowed to cool after the combustion. A dioxan-water based liquid scintillator (12 ml.) was then injected into the flask and allowed to equilibrate for 15 min with the tritiated water formed in the combustion. Finally, a 10 ml. aliquot was removed with a syringe and transferred to a counting phial.

All radioactivity measurements were made in a Packard Tri-Carb Liquid Scintillation Spectrometer (Series 3,000).

RESULTS

The concentrations of etorphine found in the maternal and foetal tissues are shown in Figs. 1a and b. Data for dihydromorphine are similarly displayed in Figs. 2a and b.

When the first samples were taken 10 min after drug administration the concentration of etorphine in maternal blood had almost reached a maximum (Fig. 1a). A plateau was maintained for 1 hr, after which clearance of the blood appeared to be fairly rapid. Thus the maternal blood levels of etorphine were consistent with the known rapid onset and moderately short duration of the drug action (Blane *et al.*, 1967). Maternal blood levels of dihydromorphine were slower to reach equilibrium, the rapid early increase levelling off by 20 min but thereafter showing no tendency to decay during the 2 hr experimental period (Fig. 2a).

The concentration of etorphine in maternal brain was well above that of the blood when the first sample was taken after 10 min, and reached a peak by 15 min at more

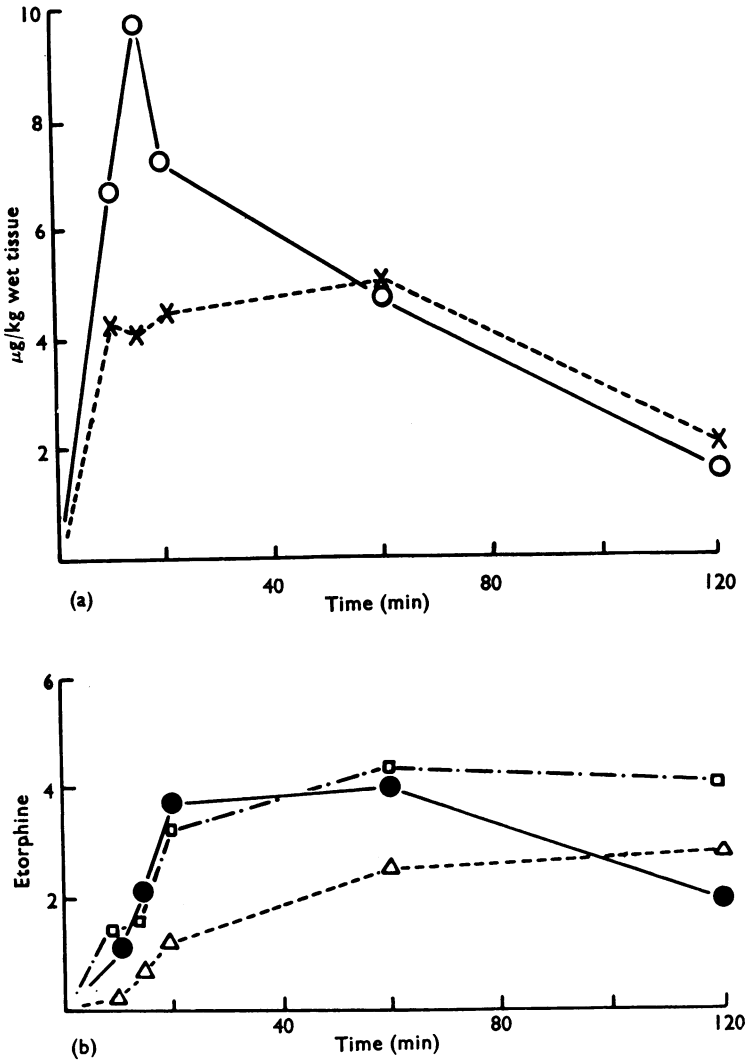

Fig. 1. Concentration of etorphine in (a) maternal (○——○=maternal brain; × - - - ×=maternal blood) and (b) foetal tissues and placentae (□ — - □=placenta; ●——●=foetal brain; △ - - - △=foetal blood) collected at intervals after administration of 12 μg/kg intramuscularly to the mothers.

than twice the corresponding blood concentration (Fig. 1a). It appeared, therefore, that etorphine was taken up selectively by brain tissue and eventually against the brain: blood concentration gradient. No such phenomenon was seen with dihydromorphine (Fig. 2a). Brain tissue concentration remained steady after the first 20 min at about 1 mg/kg despite blood levels of up to 9 mg/kg dihydromorphine.

The placental concentration of each drug closely followed that in the corresponding maternal blood (Figs. 1b and 2b). There was clear evidence, however, of a barrier

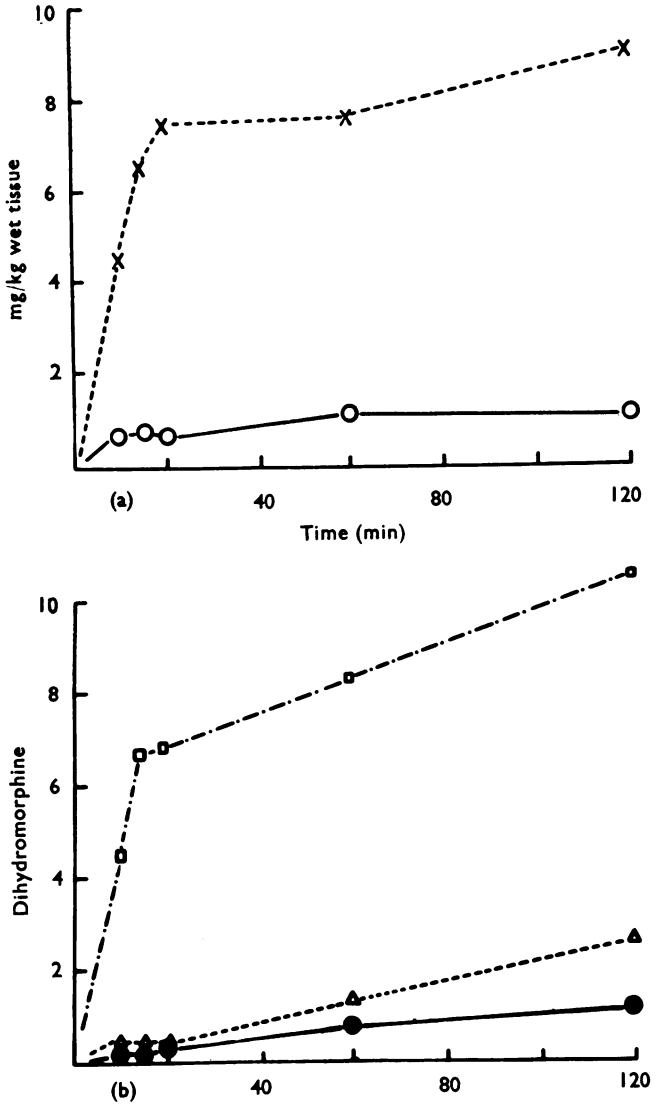

Fig. 2. Concentration of dihydromorphine in (a) maternal (× - - - × = maternal blood; ○——○ = maternal brain) and (b) foetal tissues and placentae (□-·-·□ = placenta; △- - -△ = foetal blood; ●——● = foetal brain) collected at intervals after administration of 27 mg/kg intramuscularly to the mothers.

to transport between maternal and foetal blood for both drugs. Although the rise in concentration of etorphine in foetal blood was less rapid after the first 20 min it only began to level off at the hour, at which time it was almost exactly half of the drug concentration in maternal blood. The foetal blood concentration of dihydromorphine continued to rise slowly throughout the 2 hr test period but had, even so, only reached 2.5 mg/kg in the 2 hr sample when the maternal blood concentration was 9 mg/kg.

The foetal brain, like that of the mother, took up etorphine selectively and the concentration in the foetal brain remained above that of the foetal blood at least for an hour. By contrast, and again in keeping with the maternal pattern, foetal brain levels of dihydromorphine remained always substantially lower than those of foetal blood samples collected at the same time (Fig. 2b).

DISCUSSION

No allowance was made in this investigation for the binding or metabolism of the administered labelled drugs. Thus the results were computed and expressed as if all the radioactivity present in the tissues represented unchanged drug. While this may not be a strict reflection of the true state of affairs, this study provides evidence for the general rate of transfer of the drugs from blood to brain and, more particularly, from mother to foetus.

With both etorphine and dihydromorphine the foetal blood concentrations lagged well behind those of the mother, indicating the existence of a placental barrier to their rapid transport. That the concentrations of the drugs in the placentae closely followed the maternal blood concentrations is probably a reflection of the large amount of maternal blood present in these organs and their associated deciduae.

The consistent finding with etorphine was that brain tissue, whether maternal or foetal, took up the drug with such avidity that the concentration found was above that in the circulating blood, even at the time of the first sample (10 min). The concentration ratio brain: blood of etorphine for the mothers was on average little lower than that for the foetuses (Table 1). The difference between maternal and foetal ratios was just significant (5% level) at two of the sample times only. This suggests that the maternal blood: brain barrier is penetrated by etorphine almost as readily as that of the young, but this is not so for dihydromorphine (see below). Etorphine has a higher lipid solubility than dihydromorphine; the partition coefficient carbon tetrachloride: water at pH 7.3 for etorphine = 20, and for dihydromorphine = 7 (Daglish, personal communication). This study thus provides experimental support for the hypothesis, first put forward by Boura

TABLE 1

MATERNAL AND FOETAL BRAIN: BLOOD CONCENTRATION RATIOS AT VARIOUS INTERVALS AFTER INTRAMUSCULAR ADMINISTRATION OF TRITIATED ETORPHINE OR DIHYDROMORPHINE TO THE MOTHERS

Drug	Time (min)	Concentration brain / Concentration blood		
		Mothers (A)	Foetuses (B)	B/A
Etorphine (12 μg/kg)	10	1·56	3·28	2·10*
	15	2·40	3·23	1·34
	20	1·65	2·95	1·78*
	60	0·98	1·51	1·55
	120	0·71	0·66	0·93
Dihydromorphine (27 mg/kg)	10	0·16	0·14	0·92
	15	0·10	0·33	3·22*
	20	0·09	0·45	5·24*
	60	0·15	0·46	3·05*
	120	0·12	0·41	3·29*

* Values of B/A differing significantly ($P<0·05$) from unity.

& Fitzgerald (1966) when dealing with another member of the same series of strongly analgesic drugs [N-(cyclopropylmethyl)-19-isopentylnororvinol, M320], that a lipophilic character may favour some degree of selective uptake into the central nervous system and in consequence contribute, at least in part, to very high potency—for example, etorphine ED50 for analgesia in rat equals 1.7 μg/kg subcutaneously.

By contrast with etorphine, in our experience, brain levels of dihydromorphine were always well below those of the corresponding blood level in both the mother and the foetus. Nevertheless, the ratio of the brain: blood dihydromorphine concentrations for the foetus were significantly higher than those of the mother at all times after the 10 min sample as indicated in Table 1. This broad observation is compatible with the results of Sanner & Woods (1965) and may be explained in terms of a reduced blood-brain barrier to dihydromorphine in the foetus relative to that of the mother. Such a hypothesis is consistent with clinical knowledge concerning the sensitivity of the foetal and neonatal human to known centrally acting drugs (Marx, 1961 ; Nyhan & Lampert, 1965). Way and his co-workers (Kupferberg & Way, 1963 ; Way, Costley & Way, 1965) have also commented on the relative ease with which morphine gains access to receptor sites in the central nervous system in the neonatal rat as well as in human infants.

Our results clearly indicate that the rate of transfer of etorphine across the placenta, and its accumulation in foetal brain, is much faster than that of dihydromorphine. In fact the level of etorphine in foetal brain had reached its maximum value 30 min after injection into the mothers. By contrast, and in keeping with the known longer duration of action, the tissue levels of dihydromorphine following a maternal dose which was equianalgesic with the etorphine were still rising 2 hr after injection. This consideration suggests a possible explanation for the lack of neonatal mortality after the administration of large maternal doses of morphine in an earlier study where large doses of etorphine had been lethal (Blane, 1966), assuming for the reasons already given that morphine can be equated with dihydromorphine. The young in the previous investigation were all delivered by Caesarean section only 30 min after the administration of the drug. At this time the concentration of the morphine in foetal brains would predictably have been considerably less than the expected maximum level by analogy with the results reported here for dihydromorphine, even with doses that were equianalgesic for the mothers at 30 min. Had the mothers been left until the level of morphine reached its peak in the foetal brain, which might have been 1 or 2 hr after maternal administration, it is possible that the increase in side-effects associated with the higher levels of the drug in the foetal brain would have resulted in some mortalities after delivery of the offspring. We are investigating and expect to report on this possibility at a later date.

SUMMARY

1. The concentrations of tritium-labelled etorphine and dihydromorphine in the blood and brain of pregnant rats at term have been compared with those in the foetus.

2. Although after intramuscular administration of each drug the ratio of its concentration in foetal brain to that in the blood supply was greater than that occurring in the mother, etorphine was taken up into maternal and foetal brains and crossed the placenta much more rapidly than did dihydromorphine. A further difference found between the

drugs was that etorphine accumulated in both maternal and foetal brain at concentrations greater than those in the blood.

3. It is suggested that these results may explain the differences between the neonatal toxicities of etorphine and morphine.

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