Br. J. Pharmac. (1970), **38**, 202–205.

Placental transfer of pyridostigmine in the rat

J. B. ROBERTS, B. H. THOMAS* AND ANDREW WILSON

Department of Pharmacology and General Therapeutics, University of Liverpool

Summary

1. Carbon-14 labelled pyridostigmine in single doses was given by intramuscular injection to pregnant rats, and maternal blood, foetal and placental homogenates were examined for total radioactive content, pyridostigmine and its metabolite, 3-hydroxy-N-methyl pyridinium.

2. The level of radioactivity in all tissues was highest at 15 min and fell to negligible amounts at 24 hr. The concentrations in placenta were similar to those in maternal blood, but there was some indication of retention of radioactive drug in the placenta. The concentrations in the foetus were substantially lower, but the subsequent slow decline in concentration suggests that cumulation of the drug could occur with repeated doses.

3. The percentage of metabolite in the foetus was considerably higher than in placenta and blood, and the possible reasons for this are discussed.

Introduction

Few quantitative studies of the passage of quaternary nitrogen drugs into the foetus have been reported (Moya & Thorndike, 1962). In the rabbit Edery, Porath & Zahavy (1966) investigated the placental transfer of the organophosphate antagonist, 2-hydroxyiminomethyl-N-methylpyridinium (PAM). When administered in single doses the drug was detected in the foetus only when given in large amounts (60 mg/kg). In other experiments using continuous intravenous infusion they found that the drug concentrations in foetal homogenate and maternal blood reached equilibrium after about 1 hr.

Attention has recently been focused on the placental transfer of another quaternary nitrogen compound, pyridostigmine. In a case report of neonatal myasthenia gravis, Blackhall, Buckley, Roberts, Roberts, Thomas & Wilson (1969) concluded, on the basis of plasma cholinesterase levels, clinical response, and electromyography of the baby, that placental transfer of pyridostigmine had occurred.

There is good evidence from other work in this laboratory that the fate and excretion of this drug in the rat is closely similar to that observed in patients with myasthenia gravis. The experiments described in this paper were designed to determine to what extent placental transfer of pyridostigmine and its metabolite occurs in the rat after intramuscular administration of single doses of ^{14}C-labelled pyridostigmine.

* Present address: Food and Drug Directorate, Tunney's Pasture, Ottawa 3, Canada.

Methods

^{14}C-pyridostigmine iodide labelled with ^{14}C in the methyl group of the quaternary nitrogen was supplied by the Radiochemical Centre, Amersham, and had a specific activity of 14·9 μCi/mg.

Female rats in the third week of pregnancy were allowed food and water *ad libitum* before and during the experiment. ^{14}C-pyridostigmine (0·5 mg/kg body weight) was injected intramuscularly into the hind limb and the animals were killed by decapitation at 15 min, 1, 4 and 24 hr after injection. Samples of blood were collected, the uterus was dissected and the foetuses and placentas removed, separated and weighed. Foetuses which did not respond to pinching were discarded. The samples of blood and tissue were extracted and estimated for radioactivity as previously described by Roberts, Thomas & Wilson (1965). In the present experiments, however, the extracts were freeze-dried before estimation. The mean concentrations of radioactivity were expressed as μg pyridostigmine/100 g wet weight of tissue or 100 ml. of blood.

In some experiments 3-hydroxy-N-methyl pyridinium, the metabolite of pyridostigmine, was also estimated after separation by paper electrophoresis according to the method of Husain, Roberts, Thomas & Wilson (1968).

Results

Table 1 shows that at 15 min the foetal concentration of radioactive drug is only about 3% of that in the blood; the subsequent decline in concentration is slower in the foetus than in the blood, so that at 4 hr the foetal level is 26% of the blood level. Although the placental concentration of radioactive drug is initially less than that in blood, the subsequent comparatively higher level in the former suggests some retention of radioactive drug in the placenta. By 24 hr, the concentrations in blood, placenta and foetus have declined to negligible levels.

Some observations were made of the amounts of metabolite present in the blood, placenta and foetus, and from these the percentage of metabolite at different time intervals after the administration of pyridostigmine was calculated and is shown in

TABLE 1. *Concentration of radioactivity expressed as μg pyridostigmine in the blood, foetus and placenta of rats following intramuscular administration of ^{14}C-pyridostigmine* (0·5 *mg/kg*)

Time (hr)	Blood (μg/100 ml.)	Foetus (μg/100 g tissue wet wt.)	Placenta (μg/100 g tissue wet wt.)
0·25	27·95±2·69	0·86±0·18	18·22±4·47 (5)
1	7·61±3·06	0·74±0·24	11·01±2·49 (5)
4	1·48±0·46	0·39±0·09	2·64±0·43 (3)
24	(0·20, 0·21)	(0·03, 0·06)	(0·13, 0·23) (2)

Values are means±standard deviations except at 24 hr, where individual results are recorded. The number of rats is shown in parenthesis.

TABLE 2. *Percentage of radioactivity present as metabolite in blood, foetus and placenta of rats after intramuscular administration of ^{14}C-pyridostigmine* (0·5 *mg/kg*)

Time after injection	Blood		Foetus			Placenta	
	15 min	1 hr	15 min	1 hr	4 hr	15 min	1 hr
Rat 1	16·9	27·8	79·1	78·1	92·2	14·1	33·0
2	17·1	35·8	75·2	89·0	97·5	23·7	26·0
3	19·7		62·6	74·4		11·8	41·8
Mean	17·9	31·8	72·3	80·5	94·9	16·5	33·6

Table 2. The pecentage of metabolite in blood and in placenta is very similar ; by contrast a higher proportion of metabolite was detected in the foetus.

Discussion

The results of this investigation show that when pyridostigmine is given intramuscularly in a single dose of 0·5 mg/kg to pregnant rats, the drug and its metabolite can be detected in the foetus. This evidence is at variance with the observations reported by Edery *et al.* (1966), who concluded that in the rabbit the placental transfer of another quaternary nitrogen compound, PAM, occurs only after administration of single doses about sixty times the dose of pyridostigmine used in the present experiments. This emphasizes the difficulties inherent in predicting the extent of placental transfer of drugs of this chemical nature.

The present results with pyridostigmine show that the concentrations of radioactivity in the foetus are well below those in the blood, but the concentrations in foetal tissue decline much more slowly. Myasthenic patients frequently take pyridostigmine at 4 hourly intervals, so that if the distribution of the drug in pregnant women is similar to that observed in the pregnant rat, it is possible that cumulation of this drug or its metabolite could occur in the human foetus.

The observation that the radioactivity found in the foetus is composed of 72% metabolite (and only 28% unchanged pyridostigmine) 15 min after administration is of considerable interest and merits further investigation of the mechanisms involved in the placental transfer and foetal metabolism of this drug.

The presence of a high proportion of metabolite in the foetus may be due to hydrolysis of pyridostigmine by the high concentrations of cholinesterase reported to be present in placenta (Goutier-Pirotte & Gerebtzoff, 1955 ; Gerebtzoff, 1957). From the present evidence that placental levels of metabolite are similar to those in blood it does not seem likely that hydrolysis of the drug by placental cholinesterase is an important factor. Alternatively the foetal tissues may metabolize pyridostigmine more rapidly than adult tissues or blood.

On the other hand, the high proportion of metabolite in the foetus may arise from greater permeability of the placenta to metabolite compared with pyridostigmine. There is some support for this concept, since further consideration of the results expressed in Tables 1 and 2 show that the concentration of metabolite in blood at 15 min and 1 hr are 5·00 and 2·42 μg/100 ml. respectively, in contrast to the corresponding values for foetal tissues of 0·62 and 6·60 μg/100 g. The balance of current evidence, though admittedly slender, suggests that greater permeability of the metabolite through the placenta is probably the most important factor. The results are of some interest in relation to the case of neonatal myasthenia reported by Blackhall *et al.* (1969), where the impairment in neuromuscular function may have been due to the presence of metabolite, rather than of unchanged pyridostigmine.

REFERENCES

BLACKHALL, M. I., BUCKLEY, G. A., ROBERTS, D. V., ROBERTS, J. B., THOMAS, B. H. & WILSON, A. (1969). Drug-induced neonatal myasthenia. *J. Obstet. Gynaec. Br. Commonw.*, **76**, 157–162.

EDERY, H., PORATH, G. & ZAHAVY, J. (1966). Passage of 2-hydroxyimino-methyl-N-methylpyridinium methanesulfonate to the fetus and cerebral spaces. *Tox. appl. Pharmac.*, **9**, 341–346.

GEREBTZOFF, M. A. (1957). Nouvelles recherches histochimiques sur l'acétylcholinestérase dans le placenta de cobaye. *Ann. Histochim.*, **2**, 3–10.

GOUTIER-PIROTTE, M. & GEREBTZOFF, M. A. (1955). L'acétylcholinestérase dans le placenta du cobaye. Premiers résultats de recherches histochimiques et biochimiques. *Archs int. Physiol. Biochim.*, **63**, 445–457.

HUSAIN, M. A., ROBERTS, J. B., THOMAS, B. H. & WILSON, A. (1968). The excretion and metabolism of oral ^{14}C-pyridostigmine in the rat. *Br. J. Pharmac.*, **34**, 445–450.

MOYA, F. & THORNDIKE, V. (1962). Passage of drugs across the placenta. *Am. J. Obstet. Gynec.*, **84**, 1778–1798.

ROBERTS, J. B., THOMAS, B. H. & WILSON, A. (1965). Distribution and excretion of (^{14}C)-Neostigmine in the rat and hen. *Br. J. Pharmac. Chemother.*, **25**, 234–242.

(*Received October* 3, 1969)