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Modification of transplacental distribution of salicylate in rats by acidosis and alkalosis

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1 The basis for the existence of a lower concentration of salicylate in the foetal than in the maternal blood was investigated in rats on day 20 of gestation.

2 Bolus injections of sodium salicylate were made into the mother and of [^{14}C]-salicylic acid into its foetuses and serial maternal and foetal blood samples were collected. When derived on the basis of serum salicylic acid uncorrected for differences in ionization in the maternal and foetal blood, the placental clearance was 2.2 fold greater from the foetal to maternal side than that from the maternal to foetal side.

3 The greater foetal placental clearance relative to the maternal placental clearance was not due to any active placental transfer, since there was no evidence of saturation of this process and it was not affected by pretreatment with probenecid. Moreover, salicylic acid was not concentrated by placental slices *in vitro* and its placental uptake was not affected by dinitrophenol or by cooling.

4 Maternal blood pH was 0.19 units higher than the foetal blood pH. Administration of ammonium chloride or of sodium bicarbonate into the mother increased the foetal to maternal ratio of salicylic acid from 0.6 to approximately 1.

5 It is concluded that a foetal to maternal serum salicylate concentration-ratio of less than 1 simply reflects lower ionization in the foetus than in the mother, because foetal blood pH is lower than the maternal blood pH.

Introduction

Both clinical (Gronroos *et al.*, 1981) and animal (Boulos *et al.*, 1972; Maickel & Snodgrass, 1973; Anderson *et al.*, 1980; Varma & Yue, 1983) studies show that the concentration of maternally ingested salicylate remains lower in the foetal than in the maternal blood for long periods of time. Theoretically, the ratio of foetal to maternal blood levels of any xenobiotic that crosses the placenta should attain a value of 1 or greater than 1 at some time (Dawes, 1973). A persistently lower concentration of salicylate in the foetal blood than in the maternal blood is therefore possible only if one or both of the following conditions are satisfied. (1) The foetus actively transports salicylate across the placenta against a concentration gradient or metabolizes it (Szeto, 1982). (2) The salicylate concentration difference in the foetal and maternal pool may merely reflect different degrees of ionization in the two compartments because of differences in the maternal and foetal blood pH. This study was done to investigate these possibilities. Because the fractional binding of salicylate to maternal and foetal blood proteins does not significantly differ (Anderson *et al.*, 1980; Varma & Yue, 1983), this could not account for differences in total salicylate levels in the maternal and foetal blood. Results of this study suggest that the difference in the concentration of salicylate in the maternal and foetal blood reflects differences in relative ionization in the two pools.

Methods

Experiments were done on Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) on day 20 of gestation and the presence of sperm in the vaginal washings was designated as day 0 of pregnancy. Animals had free access to laboratory rat chow and tap water.

Determination of transplacental kinetics

The pregnant rat was assumed to behave as a two-compartment open model with one maternal and one foetal pool exchanging with each other and each

to outside. In this model, drug concentrations in the maternal serum (C_m) and foetal serum (C_f) can be described by equations 1 and 2 (Varma & Ramakrishnan, 1985):

$$\frac{dC_m}{dt} = \frac{CL_{fm} C_f - CL_m C_m}{V_m} \quad (1)$$

$$\frac{dC_f}{dt} = \frac{CL_{mf} C_m - CL_f C_f}{V_f} \quad (2)$$

where CL_{fm} is the foetal placental clearance, CL_{mf} is the maternal placental clearance, CL_m is the sum of maternal placental and non-placental clearances, CL_f is the sum of foetal placental and non-placental clearances, V_m is the maternal volume of distribution, and V_f is the foetal volume of distribution.

Injections of salicylate into the mother and its foetuses and collections of blood from both were done under brief periods (2–3 min) of ether anaesthesia as described previously (Varma, 1986; Varma & Ramakrishnan, 1985). Briefly, unlabelled sodium salicylate (62 μmol kg^{-1}) was injected into the tail vein of the mother. The maternal abdomen was opened by a longitudinal incision and [^{14}C]-salicylic acid (54 mCi mmol^{-1}, New England Nuclear, Boston, Massachusetts, U.S.A.) was injected into the peritoneal cavities of all foetuses (approximately 6 nmol into each foetus) through the uterine wall; total foetal dose was 226 $\pm$ 17 nmol kg^{-1} maternal body weight. Maternal abdomen was closed by sutures. Maternal and foetal blood samples were collected serially at 1, 3, 6, 9 and 12 h. After the collection of maternal blood (0.2 ml) from the tail artery, the abdomen was again opened and 2–3 foetuses were removed starting from the ovarian end. The cut end of the uterus was ligated and the abdomen closed. Foetuses were cleaned with cotton gauze to remove the amniotic fluid after which they were decapitated and blood was allowed to drip into 0.5 ml conical polyethylene tubes. Serum salicylate was assayed as salicylic acid by h.p.l.c. (Peng *et al.*, 1978) and radiolabelled salicylic acid was quantitated by scintillation spectrometry. The concentration of radiolabelled salicylic acid was infinitely small compared with salicylic acid arising from maternal injections. At dose levels used in these studies, only salicylic acid was detected in the maternal or foetal serum.

Placental clearance rates of salicylate from the mother to foetus (CL_{mf}) and from the foetus to mother (CL_{fm}) and non-placental clearances from the mother to outside (CL_{mo}) and from the foetus to outside (CL_{fo}) were determined by fitting the solution of a two-compartment open model, assumed to represent the maternal-foetal unit, to the observed data by use of a nonlinear least square approach (Varma & Ramakrishnan, 1985; Varma, 1986), with a computer programme which can solve a general pool model with multiple injections (Ramakrishnan *et al.*, 1984).

Transplacental distribution of salicylate at different doses and after probenecid

Sodium salicylate was injected into the mother at relatively low (0.12 μmol kg^{-1}) and high (3,100 μmol kg^{-1}) doses and animals were killed 3 h later to determine the distribution of salicylic acid in the maternal and foetal serum. In other experiments, 350 μmol kg^{-1} probenecid was administered into pregnant rats 30 min before the intravenous administration of 62 μmol kg^{-1} sodium salicylate. Maternal and foetal blood samples were collected 3 h later for the assay of serum salicylic acid.

Uptake of salicylate by placental slices in vitro

Freshly removed placentas were cut into 4 pieces each and incubated in Minimum Essential Medium (MEM, Gibco, New York, U.S.A.) bubbled with a mixture of 95% oxygen and 5% carbon dioxide at 4°C or 37°C. Dinitrophenol (10 μmol ml^{-1}) was added to one set of placental slices incubated at 37°C. [^{14}C]-salicylic acid mixed with unlabelled salicylic acid was added to the incubation medium at concentrations of 0.1, 1, 10 or 100 μmol ml^{-1}. The incubation was terminated after 2 h and concentrations of salicylic acid in the incubation medium and placental tissues were determined. It was established in preliminary experiments that the placental uptake of salicylate reached a plateau after 2 h.

Effects of acidosis and alkalosis on transplacental distribution of salicylate

After the i.v. administration of 62 μmol kg^{-1} sodium salicylate, 4 mmol kg^{-1} ammonium chloride or sodium bicarbonate solution was administered orally every 30 min. Maternal and foetal blood samples were collected 3 h after the administration of salicylate.

In separate experiments sodium bicarbonate or ammonium chloride was administered orally as described above. Sodium salicylate was not injected into these animals. Maternal and foetal blood was collected into heparinized syringes for the determination of pH, Po_2 and Pco_2 by a Radio Copenhagen blood gas analyser.

Statistics

Group means were compared by one-way analysis of variance followed by comparisons of each pair of

Table 1 Maternal and foetal serum salicylic acid concentrations after simultaneous maternal and foetal injections on day 20 of gestation in rats

Serum salicylic acid	*Time (h)* 1	3	6	9	12
C_m^m (nmol ml^{-1})	281 ± 21	236 ± 17	152 ± 20	119 ± 22	93 ± 8
C_f^m (nmol ml^{-1})	129 ± 12	145 ± 7	100 ± 9	59 ± 4	43 ± 2
C_m^f (nmol ml^{-1})	207 ± 19	207 ± 17	151 ± 18	112 ± 16	99 ± 5
C_f^f (nmol ml^{-1})	209 ± 15	157 ± 10	109 ± 16	85 ± 9	75 ± 6
C_m^f/C_f^m	1.6 ± 0.2	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.7 ± 0.2

Data shown are means ± s.e., $n = 6$. Abbreviations: C_m^m—maternal concentration after maternal injection; C_f^m—foetal concentration after maternal injection; C_m^f—maternal concentration after foetal injection; C_f^f—foetal concentration after foetal injection. Concentrations after foetal injections are the observed concentrations times the ratio of the maternal to foetal dose (approximately 300 fold). The maternal dose of unlabelled salicylate was 62 μmol kg^{-1} and the foetal dose of [^{14}C]-salicylic acid was approximately 200 nmol kg^{-1}.

means in the group according to the criteria of Bonferroni (Fleiss, 1986). Comparisons between two means were done by Student's *t* test. A probability of less than 0.05 was assumed to denote a significant difference. Throughout this paper, means ± s.e. are presented.

Results

Transplacental kinetics

The concentration of salicylate was higher in the maternal serum than in the foetal serum at all times after the maternal and from 3 to 12 h after the foetal injections (Table 1). As predicted by the solution of the model (Varma & Ramakrishnan, 1985), the relationship between the maternal concentration of salicylate after foetal injection to foetal concentration after maternal injection remained relatively constant during the course of an experiment. Figure 1 depicts the computer plot of one experiment which yielded values closer to mean values for all experiments.

Based on total salicylic acid concentration not corrected for differences in ionization in the maternal and foetal serum, the placental clearance of salicylate from the foetal to maternal side was 2.2 ± 0.6 times greater than that from the maternal to foetal side. The non-placental foetal clearance was zero (Table 2).

Effects of different doses and probenecid on the transplacental distribution of salicylate (Table 3)

Increasing the maternal dose of salicylate from 0.12 to 3,100 μmol kg^{-1} did not significantly alter the ratios of the foetal serum to maternal serum salicylate concentrations, which ranged from 0.6 ± 0.06 to 0.66 ± 0.03. Likewise, oral administration of

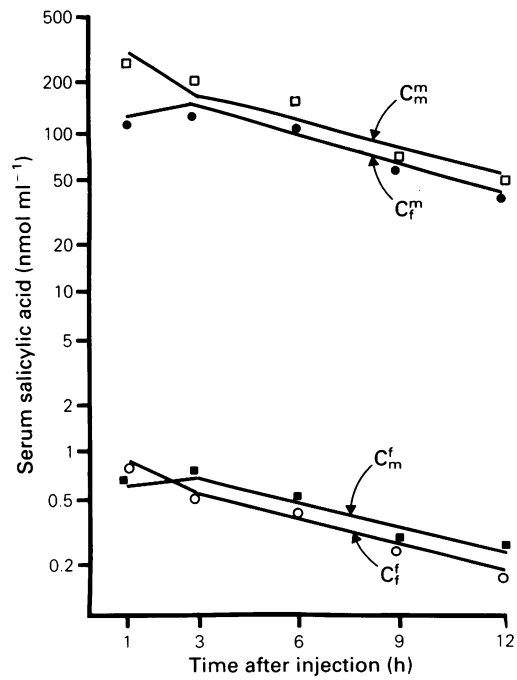

Figure 1 A plot of the maternal and foetal serum salicylic acid concentration after i.v. injection of sodium salicylate (62 μmol kg^{-1}) into the mother and i.p. injections of [^{14}C]-salicylic acid (190 nmol kg^{-1} maternal body weight) into its foetuses. C_m^f—maternal concentration after foetal injection; C_m^m—maternal concentration after maternal injection; C_f^f—foetal concentration after foetal injection; C_f^m—foetal concentration after maternal injection.

Table 2 Transplacental kinetics of salicylate in rats on day 20 of gestation

Kinetic derivations	
Maternal V_m (ml kg^{-1})	234 ± 16
Foetal V_f (ml kg^{-1})	31 ± 1*
CL_{mf} (ml $kg^{-1} h^{-1}$)	60 ± 11
CL_{fm} (ml $kg^{-1} h^{-1}$)	105 ± 12*
CL_{mo} (ml $kg^{-1} h^{-1}$)	28 ± 7
CL_{fo} (ml $kg^{-1} h^{-1}$)	0*
CL_{fm}/CL_{mf}	2.2 ± 0.6

Data shown are means ± s.e. ($n = 6$) and are derived from total serum salicylic acid concentrations not corrected for differences in ionization in maternal and foetal blood. Abbreviations: CL_{mf}—placental clearance from mother to foetus; CL_{fm}—placental clearance from foetus to mother; CL_{mo}—maternal non-placental clearance; CL_{fo}—foetal non-placental clearance. Volumes of distribution (V_m and V_f) and clearances are in terms of maternal body weights. Doses of sodium salicylate into the mother and of [^{14}C]-salicylic acid into its foetuses were 62 μmol kg^{-1} and approximately 200 nmol kg^{-1} maternal body weight, respectively.
* Significantly ($P < 0.05$) different from the immediate top value.

350 μmol kg^{-1} probenecid 30 min before the injection of salicylate did not exert a significant effect on the ratio of foetal to maternal serum salicylic acid.

Salicylate uptake by placental slices in vitro (Table 4)

Placenta did not concentrate salicylic acid and placental tissue concentration was approximately 70% of the media concentrations (0.095–96.6 μmol ml^{-1}). The ratio of the tissue to media concentration of salicylic acid was not significantly altered by dinitrophenol or by lowering the temperature of the tissue bath to 4°C.

Effects of NH_4Cl and $NaHCO_3$ on blood pH, Po_2 and Pco_2 (Table 5)

Foetal blood pH and Po_2 were significantly lower and Pco_2 higher than the corresponding values for the maternal blood. Administration of NH_4Cl significantly decreased both the foetal and maternal blood pH; perhaps the dose was too high causing respiratory depression as reflected by the changes in blood Po_2 and Pco_2. Maternal but not the foetal blood pH significantly increased after administration of $NaHCO_3$.

Effect of acidosis and alkalosis on the transplacental distribution of salicylate (Table 3)

After repeated oral administrations of NH_4Cl or $NaHCO_3$, the ratio of foetal serum to maternal serum salicylic acid concentrations increased significantly and approached unity. In order to ascertain more about the effects of ammonium chloride and sodium bicarbonate on the ratios of foetal to maternal serum salicylic acid concentrations, blood samples were collected before as well as 5 min and 6 h after the end of repeated administrations of either ammonium chloride or sodium bicarbonate. The ratio of salicylic acid in the foetal blood to that in the maternal blood rose from 0.59 ($n = 3$) to 1.1 at the end of 4 repeated doses of ammonium chloride;

Table 3 Effects of different treatments on the distribution of salicylate in the maternal and foetal serum of rats on day 20 of gestation

Treatment	*Salicylate dose* (μmol kg^{-1})	n	*Serum salicylic acid* *Maternal (MS)* (nmol ml^{-1})	*Foetal (FS)* (nmol ml^{-1})	*FS/MS*
None	0.12	4	0.35 ± 0.02	0.21 ± 0.03	0.61 ± 0.05
None	62	9	273 ± 21	168 ± 8	0.60 ± 0.04
None	3,100	4	3295 ± 150	2181 ± 81	0.66 ± 0.03
Probenecid	62	4	379 ± 26	181 ± 11	0.58 ± 0.03
NH_4Cl	62	5	342 ± 32	336 ± 24	0.98 ± 0.07**
$NaHCO_3$	62	5	120 ± 20*	124 ± 22	1.12 ± 0.15**

Serum salicylic acid concentrations correspond to 3 h after i.v. injection of sodium salicylate. Probenecid (350 μmol kg^{-1}) was administered orally 30 min before the injection of sodium salicylate, NH_4Cl and $NaHCO_3$ were administered orally at 30, 60, 90, 120, 150 and 180 min after salicylate injection, each time at a dose level of 4 mmol kg^{-1}
* Significantly ($P < 0.05$) different from all other values in the same column; ** not different from each other but significantly ($P < 0.05$) different from all other values in the same column.

Table 4 Uptake of salicylic acid by rat placental slices *in vitro*

Salicylic acid added (M)	*DNP*	*Temp.* (°C)	*Salicylic acid concentration* *Tissue (T)* (μmol g^{-1})	*Buffer (B)* (μmol ml^{-1})	*T/B*
10^{-4}	Absent	37	75 ± 3	95 ± 1	0.78 ± 0.03
10^{-4}	Present	37	75 ± 6	98 ± 3	0.77 ± 0.08
10^{-3}	Absent	4	530 ± 50	940 ± 20	0.80 ± 0.05
10^{-3}	Absent	37	696 ± 30	976 ± 7	0.71 ± 0.03
10^{-3}	Present	37	710 ± 10	1000 ± 30	0.72 ± 0.03
10^{-2}	Absent	4	5200 ± 60	9700 ± 20	0.81 ± 0.04
10^{-2}	Absent	37	7730 ± 131	9860 ± 68	0.69 ± 0.03
10^{-2}	Present	37	7045 ± 246	9850 ± 89	0.71 ± 0.02

Data are means ± s.e. of 4 experiments each of which was done in duplicate. Placentas from one animal (day 20 of gestation) were used for 1 determination under all of the above experimental conditions. There was no significant difference in the placental tissue/buffer ratios (T/B) under different experimental conditions. DNP—dinitrophenol concentration was 10 μmol ml^{-1}.

6 h after the last dose of ammonium chloride, the ratio declined to 0.78. Likewise, the foetal-maternal serum salicylic acid ratio rose from 0.51 ($n = 3$) to 1.17 at the end of 4 repeated doses of sodium bicarbonate and then declined to 0.72 after a lapse of 6 h.

Discussion

The main purpose of these studies was to find out the mechanism responsible for a relatively lower salicylate level in the foetal than in the maternal blood found by several workers (Boulos *et al.*, 1972; Maickel & Snodgrass, 1973; Anderson *et al.*, 1980; Gronroos *et al.*, 1981; Varma & Yue, 1983). Rat foetuses do not metabolize salicylate (Varma & Yue, 1983). Therefore, such a gradient could exist if there is an active placental transport of salicylate from the foetal to maternal side as in the case of dexamethasone (Varma, 1986) and triamterene (McNay & Dayton, 1970). Alternatively, the differences in salicylate concentrations between the maternal and foetal sera may merely reflect a greater relative ionization in the maternal than in the foetal blood due to the higher maternal blood pH without the existence of any true gradient of non-ionized moiety of salicylate.

Based on total salicylic acid concentrations, the placental clearance rate from the foetal to maternal side was 2.2 fold greater than that in the opposite direction. This would indicate that placenta actively transports salicylate from the foetal to maternal side. However, several observations made in the present study show that this is not the case.

An increase in the dose of sodium salicylate did not significantly change the ratio of foetal to maternal serum salicylic acid concentration indicating lack of saturability of the transport process. Probenecid which competitively inhibits renal tubular salicylate transport did not modify the relationship between

Table 5 Effects of NH_4Cl and $NaHCO_3$ on maternal and foetal blood pH, Po_2 and Pco_2 of rats on day 20 of gestation

Measurements	*Saline*	*Treatment* *NH_4Cl*	*$NaHCO_3$*
Maternal pH	7.28 ± 0.02[a]	6.85 ± 0.04[a, b]	7.46 ± 0.02[a, b]
Foetal pH	7.09 ± 0.03	6.89 ± 0.01[a]	7.08 ± 0.12
Maternal Po_2	32.90 ± 2.91[a]	15.20 ± 0.80[a, b]	31.25 ± 3.21[b]
Foetal Po_2	22.60 ± 0.02	25.70 ± 1.30	15.25 ± 2.27[b]
Maternal Pco_2	52.30 ± 1.43[a]	85.05 ± 8.13[b]	71.15 ± 2.78[a, b]
Foetal Pco_2	64.62 ± 0.83	76.31 ± 9.76	107.12 ± 1.03[b]

[a] Denotes significant ($P < 0.01$) difference from the corresponding foetal value (immediate bottom), and [b] denotes significant ($P < 0.01$) difference from the value in the same row for the saline-treated animal; data are means ± s.e., $n = 4$–5.

maternal and foetal salicyclic acid levels. Placental slices *in vitro* did not accumulate salicylic acid; indeed at all concentrations the ratio of tissue to medium concentration of salicylate corresponded to the ratio of tissue water to total tissue mass. The concentration of placental tissue salicylate was neither modified by dinitrophenol nor by lowering the temperature of the incubation medium. Finally, if transplacental kinetics were calculated on the basis of non-ionized moiety assuming foetal blood pH to be 0.1 to 0.2 units lower, the clearance ratios from the mother to foetus and that from the foetus to mother became approximately equal. In conclusion, the differences in the placental clearance rates derived on the basis of total salicylate concentrations are misleading and in this sense present a limitation of the model for a study of active placental transfer (Varma & Ramakrishnan, 1985; Varma, 1986) of weak acids and weak bases. McNay *et al.* (1969) had proposed earlier that *para*-aminohippuric acid is not actively transported by sheep placenta.

Administration of either ammonium chloride or of sodium bicarbonate significantly increased the ratio of the foetal to maternal serum salicylic acid concentration. This would strongly suggest that the maternal to foetal gradient of salicylic acid, which is apparent on the basis of total serum salicylic acid concentrations, is merely a reflection of different degrees of ionization in the maternal and foetal blood. The pK_a of salicylic acid, the form in which salicylates exist in the serum, is 3.0. In accordance with the Henderson-Hasselbalch equation the relative concentrations of the ionized and nonionized salicylic acid will be:

$$pH = pK_a + \log [\text{ionized salicylic acid/non-ionized salicylic acid}].$$

At all pH ranges observed in this study most of the salicylic acid will exist in an ionized form and the non-ionized fraction will be infinitely small. Therefore, the total concentration in both the maternal and foetal blood is essentially a reflection of the ionized fraction. In so far as the ionized fraction is trapped on either side of the placenta, the relative concentrations of total salicylic acid are determined by the pH. At the mean maternal blood pH of 7.28 and foetal blood pH of 7.09 as found in these studies, the ratio of ionized fraction in the foetal blood to that in the maternal blood will be 0.65, which is very close to the experimentally determined ratio.

The administration of ammonium chloride changed the maternal and foetal blood pH to 6.85 and 6.89, respectively. Theoretically at this lower pH, the ratio of foetal to maternal serum salicylic acid should achieve a value of 1.09, which is close to the observed ratio of 0.98.

Although the transplacental distribution of salicylate is consistent with a pH gradient that exists in saline or ammonium chloride treated animals, the effect of sodium bicarbonate is somewhat puzzling. Sodium bicarbonate was not effective in changing the foetal pH and increased the maternal pH. This should have further decreased the foetal to maternal serum salicylic acid ratio. However, the opposite was observed. It would seem that during maternal alkalosis salicylic acid is eliminated by the maternal kidney more rapidly than it is being transferred from the foetus into the mother, leading to a relatively higher foetal to maternal ratio of salicylate. On the other hand, changes in blood pH after the administration of sodium bicarbonate and ammonium chloride could change the fractional binding of salicylate to maternal and foetal plasma proteins, and in turn its transplacental distribution. The present study does not exclude this possibility. It may be added that despite the pH differences in the maternal and foetal blood under physiological conditions, the binding of salicylate to maternal and foetal plasma proteins does not differ significantly (Anderson *et al.*, 1980; Varma & Yue, 1983).

Although the effect of acidosis and alkalosis on the transplacental distribution of salicylate has not been described, studies with other agents support the conclusion reached above. For example foetal-maternal lignocaine ratio increased from 0.76 to 1.21 when sheep foetuses were made acidotic (Biehl *et al.*, 1978). Reynolds (1979) presented theoretical arguments with bupivacaine as an example that maternal-foetal concentration differences of weak acids and bases are mostly due to the lower pH of the foetal blood than of the maternal blood.

The results of the present study suggest that reported discrepancies in the ratio of foetal to maternal concentration of salicylate from 0.22 (Anderson *et al.*, 1980) to >1 (Levy & Garrettson, 1974) are most probably due to differences in acid/base balance during different experimental conditions.

In conclusion, the present study demonstrates that the relatively higher concentration of salicylate in the mother than in the foetus reflects pH-dependent differences in ionization in the maternal and foetal units and does not represent the existence of any true gradient.

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