The effects of smoking on placental membranes

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Received 7 March 2001; received in revised form 4 September 2001; accepted 12 September 2001

Abstract

Ingested tobacco smoke contains many toxic components that may harm the membrane compartments of the human placenta. The effects of maternal smoking on placental membrane structure are examined by stereological methods and related to smoking habit. A significant decrease in fetal capillary volume and increase in the diffusion distance across the trophoblastic epithelium is observed. The stereologically-determined structural variables are used to estimate both total and partial oxygen diffusive conductances. The total diffusive conductance is found not to alter with smoking; however, changes in partial conductances and the haematocrits of both maternal and fetal blood, indicate hypoxic stress is associated with smoking. However, a component of the placental membranes responsible for the transport of alanine is shown to be altered. An increase in the sodium-dependent transport of alanine across the microvillous border membrane of the placenta is observed in tissues derived from mothers who smoke. This may be an adaptive response to a deficient supply of this amino acid to the placenta.

Keywords: Biological membranes; Diffusion; Facilitated transport

1. Introduction

Theoretical and practical studies on synthetic polymers conducted by such workers as P. Meares have provided many valuable insights into the properties and behaviours of membrane systems. Whilst much of this work has found immediate application to man-made membranes and related industrial processes, such studies have also contributed significantly to our understanding of biological systems with consequent benefit to medical science. However, the analysis of transport phenomena in bio-membranes involves a number of problems that are not normally present in studies on synthetic systems. Biological structures are often highly heterogeneous and capable of altering in response to external stimuli. The membranes may not be fully accessible to the investigator and, frequently, it is not possible to define, let alone control, all the variables associated with the transport processes occurring within them. The major thrust of this paper will be to describe specific strategies that have been used to deal with these problems.

It is now well established that maternal smoking has a detrimental effect on fetal growth [1]. Although
the mechanisms underlying this phenomenon are not yet fully understood, there are good grounds for considering that they may involve the placenta. The placenta operates at the interface between the maternal and fetal blood circulations and, hence, is the organ by which nutrients are passed between the mother and fetus prior to birth. A failure in its function would therefore lead to impaired fetal growth.

Maternal blood is carried to the fetus via the uterine blood vessels. From here, maternal blood comes into direct contact with fetal tissue by perfusing the intervillous space. The outer surface of fetal tissue consists of what is essentially a single layer of epithelium termed syncytiotrophoblast. The syncytiotrophoblast forms the principal tissue barrier between the maternal and fetal blood streams. It is a true syncytium in that it grows by the recruitment of post-mitotic cells (cytotrophoblasts) which fuse with it and form a multinucleated continuum [2]. Two separate membrane domains bound the trophoblastic syncytiotum, the outer being the microvillous border and the inner the basal border. Within the placenta, fetal blood passes from the umbilical cord through blood vessels that ultimately supply the capillaries of the villi, the latter being frond-like extensions that extend into the intervillous space. The villi are washed by the maternal blood and thus they provide the site of nutrient exchange between maternal and fetal tissues. The fetal capillaries are surrounded by a loose array of connective tissue (stroma) and this in turn is bounded by the syncytiotrophoblast.

A nutrient must therefore cross a series of membranes in its passage between the maternal and fetal blood streams, any of which could provide the rate-determining step. If the nutrient readily crosses the membranes, the blood streams could become rate-determining instead. Different barriers are likely to be involved with different nutrients depending upon their physical and chemical properties. Oxygen, being lipophilic, is more likely to be limited by blood flow (or, to be more precise, less likely to be limited by diffusion), whilst an amino acid such as alanine, being an important hydrophilic nutrient, is more likely to be membrane-limited. There are further complications in this process in that a species such as oxygen is chiefly carried in the blood stream by haemoglobin, this protein in turn being contained by the plasma membrane of the red blood corpuscle. Therefore, delivery of oxygen to the placenta will be governed in part by the haemoglobin content of the maternal blood, and in part by the processes by which oxygen dissociates from the haemoglobin and passes across the cell membrane into the plasma. Similar considerations will apply to its transport in the fetal blood stream. With regards to alanine, it is likely to cross the placental membranes by associating with one or more membrane-bound proteins which act as carriers. The chemical interaction of the amino acid with the carrier molecule is therefore of importance, and this can be complicated further by the fact that transport may be modified by the presence of a third component such as sodium. It is known from other systems that alanine can be transported across bio-membranes by either sodium-independent or sodium-dependent carriers.

Tobacco contains a wide variety of compounds that are potentially toxic to human tissue, in particular carbon monoxide, polycyclic aromatic hydrocarbons, and cadmium. The potential effects of smoking will be in proportion to the severity of exposure. In this study, exposure was estimated by patient questionnaire, and by assay of cotinine (a major metabolite of nicotine) in the maternal blood stream. The impact of smoking on the placental tissue was assessed by measuring the activity of placental ethoxyresorufin O-deethylase (EROD). This enzyme is dependent on cytochrome P450 and is induced by cigarette smoking [3]. As an alternative assessment of the impact of smoking, concentrations of cadmium in the placental tissues were measured.

Direct studies on the human are strictly limited by ethical constraints. The placenta is thus largely inaccessible to direct study of transport phenomena using in vivo methods. In recent studies by the authors [4,5], structural quantities (vascular volumes, exchange surface areas and tissue diffusion distances) have been estimated by stereological analyses of placental samples drawn from non-smoking and smoking women. These quantities are then combined with previously-published physicochemical quantities to permit an estimation of the effects of maternal cigarette smoking on transplacental oxygen diffusion. Although not a direct measurement of oxygen transport it will be shown that rational conclusions can be reached from these data that relate to the placental diffusive conductance and hence allow conclusions to be drawn concerning the physiological effects of smoking on fetal oxygen status.
Studies in relation to intrauterine growth retardation (IUGR) [6] suggest that the microvillous border membrane may provide the rate-determining step for the transport of amino acids such as alanine across the placenta. We have therefore examined the uptake of this amino acid across microvillous border membranes obtained from placentas drawn from non-smoking and smoking women [7]. This latter study has utilised the fact that the microvillous membrane may be extracted in pure form from the placenta and then formed into vesicles that expose the outer surface of the membrane to the suspension medium (‘right side out’ vesicles). The use of such vesicles thus allows investigation of the effects of maternal smoking on the uptake kinetics of this amino acid.

2. Experimental

2.1. Tissue collection

Caucasian women were recruited at approximately 13 weeks of gestation and their declared smoking habits assessed by questionnaire. A further interview was completed at 34 weeks gestation when a blood sample was collected and stored at −140 °C for cotinine analysis. Placentae were obtained after spontaneous vaginal delivery or caesarian section. Immediately following birth, samples were collected of umbilical vein and maternal blood and haematocrits determined in triplicate from each. Placental volume was estimated from trimmed placental weight divided by tissue density. Tissue samples were taken for stereology as described below. Crude homogenates of placental tissue were prepared and stored at −140 °C for EROD analysis. Placental scrapings for uptake studies were prepared by blunt dissection and placed on ice. Microvillous border plasma membrane was prepared within 90 min of delivery from these scrapings using the protocol described below.

2.2. Stereology

The principles of the stereological approach employed have been reviewed by Mayhew [8]. Random samples of tissue were taken and diced into approximately 1 cm³ cubes. Tissue pieces were fixed by immersion in 10% paraformaldehyde and then embedded in paraffin wax at chance orientations. Tissue sections were cut at a nominal thickness of 4 μm and stained by the Martius Scarlet Blue method [9] for light microscopical sampling. Systematic random fields of view were analysed as micrographs (final linear magnification 200×) and as projected colour slide transparencies (4000×). All magnifications were calibrated using external scale standards.

Volumes of intermediate and terminal villi (Vitv) and maternal intervillous space (Vivs) were estimated by superimposing a transparent lattice of test points at random positions on each micrograph and counting those points hitting sectional images of chorionic villi and intervillous space, respectively. The numbers of points hitting each tissue compartment, divided by the total number of points hitting the placenta itself, provided unbiased estimates of the relative volumes of the placenta occupied by those compartments. These relative volumes, multiplied by the placental volume, then yielded Vitv and Vivs. In the same fashion, the volumes of trophoblast (Vtro), stroma (Vstr) and fetal capillaries (Vfc) were estimated by projecting slides onto a lattice drawn on white card, counting the appropriate intersections, dividing each by the total number of lattice points and multiplying by the total villous volume. Surface area densities within the placenta or its villi were obtained by projecting slide transparencies onto lattices drawn on white card, counting the appropriate intersections, dividing each by the total number of lattice points and multiplying by the total villous volume. The capillary/villous length ratio (Lfc/Litv) was estimated by extrapolation from corresponding volumes and surface areas. Harmonic mean thicknesses for trophoblastic (Thtro) and stromal (Tstr) components of the villous membrane were determined by using test lines to identify random sites from which to measure orthogonal intercept lengths across the relevant tissue layer. Harmonic thickness is preferable to arithmetic mean thickness because it affords greater weight to thinner areas of membrane (i.e. those areas over which oxygen diffuses more easily).
2.3. Diffusive conductances

The model described by Mayhew et al. [10,11] was employed to estimate oxygen diffusion. The route by which a molecule diffuses between the maternal and fetal haemoglobin is divided into six serially arranged compartments: maternal red blood corpuscles, maternal inter villous blood plasma, villous trophoblast, villous stroma (including fetal capillary endothelium), fetal capillary blood plasma, and fetal red blood corpuscles. Diffusive conductances \( (D_\text{ij}) \) across each of these compartments were estimated from the volumes, surface areas and harmonic mean thicknesses of the tissues obtained from the stereology, together with the oxygen–haemoglobin mean thicknesses of the tissues obtained from the volumes, surface areas and harmonic mean thicknesses of the tissues obtained from the stereology, together with the oxygen–haemoglobin reaction rates in maternal and fetal blood \( (\Theta_p, \Theta_b) \), and the Krogh diffusion coefficients for oxygen in maternal and fetal plasma \( (K_p, K_b) \), trophoblast and stroma \( (K_{\text{ts}}) \). Partial conductances for maternal \( (D_{\text{pm}}) \) and fetal \( (D_{\text{ft}}) \) red blood corpuscles were thus \( V_{\text{rc}} \times \Theta_p \) and \( V_{\text{rc}} \times \Theta_b \). Conductances for maternal \( (D_{\text{mp}}) \) and fetal plasma \( (D_{\text{fp}}) \) were estimated by \( K_p (S_{\text{mp}} + S_{\text{hmp}})/2T_{\text{hmp}} \) and \( K_b (S_{\text{fb}} + S_{\text{hfp}})/2T_{\text{hfp}} \). For trophoblast \( (D_{\text{bt}}) \) and stroma \( (D_{\text{st}}) \), the estimators were \( K_p (S_{\text{b}} + S_{\text{htb}})/2T_{\text{htb}} \) and \( K_b (S_{\text{st}} + S_{\text{hts}})/2T_{\text{hts}} \), respectively. The total diffusive conductance \( (D_p) \) was estimated as \( 1/D_p = \sum (1/D_{\text{ij}}) \). The following values were used for the physical constants, \( \Theta_p = 12.8 \text{ ml/min/kPa}, \quad K_p = 24 \times 10^{-7} \text{ cm}^2/\text{min/kPa}, \quad \text{and} \quad K_b = 17.3 \times 10^{-7} \text{ cm}^2/\text{min/kPa} \) [10].

2.4. Assays

Cotinine was assayed by the method of Perkins et al. [12]. Cotinine was extracted from plasma using C18 solid phase cartridges (Millipore, Watford, UK) evaporated under nitrogen and reconstituted in water before analysis by high performance liquid chromatography. Recovery from spiked plasma (200 ng/ml) was 100% (coefficient of variation: 12.6%). The standard curve was linear up to 800 ng/ml, and the limit of detection was 30 ng/ml.

EROD was assayed using microsomes prepared from crude placental homogenates by calcium precipitation. EROD activity was then determined by the method of Burke and Mayer [13]. The synthesis of the metabolite resorufin from ethoxyresorufin was measured by fluorimetry in the presence of placental microsomal protein. The limit of detection for EROD activity was approximately 2 pmol resorufin/min/mg protein and the standard curve was linear across all concentrations used.

Protein assays using the Coomassie Blue Dye binding method (Bio-Rad protein assay kit) were performed on samples of homogenate and microvillous plasma membranes. Protein yields were estimated as the amount of protein present in the microvillous plasma membranes expressed as a percentage of homogenate protein.

Alkaline phosphatase (ALP) assays were performed by hydrolysis of p-nitrophenyl phosphate (SIGMA ALP Kit No. 245).

Placental cadmium concentration was determined by inductively coupled mass spectrometry (ICPMS). Randomly sampled pieces of placental tissue were washed in balanced saline until all traces of blood had been removed. Following this the tissue was blotted dry, minced, and freeze dried. It was next digested in a microwave oven and then dissolved in 16% v/v nitric acid. Indium was added to each sample to provide internal calibration. Blanks, and samples spiked with cadmium were also prepared. ICPMS was performed using a VG plasmaquad 1 (FI Elemental, UK). Sample cadmium concentrations were calculated against an internal calibration curve. Recovery was 105 ± 2% and the standard curve linear over the 0.1–1000 ng/g wet weight range with a detection limit of 1 pg/g wet weight.

2.5. Uptake studies

Uptake of alanine by microvillous border vesicles (MBV) was measured using the protocol described by Page et al. [14]. Microvillous plasma membranes were prepared from placental scrapings by stirring the tissue on an ice-bath first in 100 mM CaCl₂, then in isotonic-buffered (Tris) saline, and then filtered through fine gauze. The protein ‘homogenate’ thus obtained was centrifuged successively at 88 × g for 10 min, and 10,000 × g for 20 min and the pellets discarded, and then spun at 110,000 × g for 30 min. The final pellet, which represents the microvillous plasma membrane was resuspended in 150 mM NaCl, 2 mM Tris–Cl pH 7.2.

ALP is an enzyme located only in the microvillous border and hence can be used as a marker of...
membrane purity. Enzyme activities in both homogenate and microvillous plasma membranes were measured and the results expressed as international units per milligram protein. The microvillous plasma membranes were stored at $-140^\circ$C until required.

MBV were produced from the microvillous plasma membranes by thawing the membranes on ice, suspending them in isotonic-buffered KCl as required, and passing the resultant suspension repeatedly through a 19 gauge needle.

Uptake of alanine was measured by incubating the vesicles at 22°C in solutions containing different concentrations of alanine (range 1.6–16 mM) in 145 mM NaCl or 145 mM KCl and 2 mM Tris–HEPES buffer pH 7.4. Reactions were terminated by addition of ice-cold isotonic buffer followed by rapid filtration through 0.45 μm cellulose based filters (Millipore, Watford, UK). Alanine uptakes were obtained from the activities of tritiated alanine in the bathing solution and in the vesicles. Non-specific binding was estimated from the activities of filters exposed to vesicle free labelled alanine solutions.

2.6. Statistical methods

All data were expressed as group means and standard errors of means (S.E.M.) and statistical analysis of data was undertaken using SigmaStat software (Jandel Scientific, Erkrath, Germany). For morphometric measurements and uptakes means were compared using analyses of variance and either one or two tailed Student’s $t$-tests as appropriate.

### Table 1

Correlations between markers of smoking

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$N$</th>
<th>$R$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cotinine (ng/ml) vs. self-declared smoking rate (cigarettes per day)</td>
<td>86</td>
<td>0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EROD activity (pmol resorufin/min/mg protein) vs. self-declared smoking rate (cigarettes per day)</td>
<td>97</td>
<td>0.41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EROD activity (pmol resorufin/min/mg protein) vs. plasma cotinine (ng/ml)</td>
<td>91</td>
<td>0.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cadmium (ng/g wet weight) vs. self-declared smoking rate (cigarettes per day)</td>
<td>48</td>
<td>0.10</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* $N$ = Number of placenta; $R$ = Correlation coefficient; $P$ = Probability of failure of null hypothesis.

3. Results and discussion

3.1. The impact of smoking

The correlations between markers of smoking and self-assessed smoking rates are summarised in Table 1. Self-declared smoking rates ranged from 0 to 40 cigarettes per day. Group mean cotinine levels of 0.62 ± 0.52 ng/ml were recorded in confirmed non-smokers, whilst the corresponding figures for confirmed smokers were 131 ± 18.8 ng/ml. Plasma cotinine levels correlated positively both with placental EROD activity and with placental cadmium concentration.

The close correlations between self-declared smoking rates and concentrations of plasma cotinine shows that in this study there were very few 'deceivers' in relation to the subjective assessments of smoking. The significant correlations between EROD and both plasma cotinine and self-declared smoking rate reflects the impact of ingested tobacco smoke on placental metabolism. Cigarette smoking is known to induce placental EROD activity [15]. This is catalysed by the CYP1A family of cytochrome P450 [16]. The induction most likely results from the action of polycyclic aromatic hydrocarbons, such as benz[a]pyrene which are present in tobacco smoke.

The cadmium content of tobacco leaf varies with the geographic location of the plant but, typically, about 1 μg of cadmium is present in the tobacco contained within a cigarette. The correlation of tissue cadmium and self-declared smoking rate indicates that
Harmonic mean thickness of stroma, $T_{\text{str}}$ (cm)
Volume of trophoblast, $V_{\text{tro}}$ (cm$^3$)
Volume of villi, $V_{\text{itv}}$ (cm$^3$)
Volume of fetal capillaries, $V_{\text{fc}}$ (cm$^3$)
Surface area of villi, $S_{\text{itv}}$ (cm$^2$)
Surface area of fetal capillaries, $S_{\text{fc}}$ (cm$^2$)
Harmonic mean thickness of trophoblast, $T_{\text{htro}}$ (µm)
Harmonic mean thickness of stroma, $T_{\text{hstr}}$ (µm)

3.2. Placental morphology

Table 2 summarises the morphometric measurements made on placenta derived from 17 non-smokers and 22 smokers, all singleton pregnancies. The $V_{\text{itv}}$ was significantly smaller in smokers compared to non-smokers whilst $T_{\text{htro}}$ was significantly higher in smokers than in non-smokers. Significant differences ($P < 0.05$) were also observed in the ratios $V_{\text{fc}}/V_{\text{itv}}$ (non-smokers: 0.363±0.009, smokers: 0.396±0.006); $V_{\text{tro}}/V_{\text{itv}}$ (non-smokers: 0.202 ± 0.015, smokers: 0.163 ± 0.010); $S_{\text{fc}}/S_{\text{itv}}$ (non-smokers: 0.130 ± 0.009, smokers: 0.167 ± 0.013).

These results indicate that there is significant decrease in fetal capillary volume associated with smoking, a finding in agreement with other workers [17]. However, there were no significant differences in villous surface area or the villus/capillary length ratio $L_0/L_{\text{itv}}$. This suggests that the reduction in capillary volume is due to a decrease in the vessel calibre rather than its length. An increase in the diffusion distance across the trophoblast was also observed, but without a change in trophoblast volume. This could involve a thickening of the trophoblast basal lamina, or lamina duplication. Alternatively, it could also result from an impairment of the normal processes of cytotrophoblast differentiation to syncytotrophoblast or a reduced extrusion of syncytial trophoblast fragments into the intervillous space due to an inhibitory effect of smoking on trophoblast apoptosis [5].

3.3. Placental oxygen diffusive conductance

It is technically extremely difficult to provide a direct measure of oxygen transport across the placenta. In the case of the human where the placenta is effectively inaccessible up to the moment of birth the estimation of the oxygen diffusive conductance from stereologically-determined parameters and physical constants thus provides the only practicable way at present of arriving at this quantity. This poses the problem as to how what is essentially an anatomical value relates to the effective or ‘physiological’ oxygen diffusive conductance. The placenta as an active organ will metabolise oxygen but this is not allowed for in the present calculations. In addition, there are likely to be vascular shunts and perfusion inequalities present that also are not included in the present model. The morphometric estimation of diffusive conductance can therefore be looked upon as an index of the potential for oxygen diffusion afforded by the physical dimensions of the placenta rather than an estimate of the actual physiological quantity [18]. Despite this shortcoming it has proved on many occasions to be an extremely useful quantity for comparing normal to abnormal pregnancies and in this regard to provide some guidance as to the magnitude of the physiological conductance [5].

The total oxygen diffusive conductance of placentae from non-smokers, $D_0$, was not significantly different from that obtained from smokers (non-smokers: 16.3±1.89 ml/min/kPa, smokers: 16.3±0.095 ml/min/kPa). The same result applied to specific conductances, that is total conductances normalised for birthweight
between smoking and non-smoking groups.

In the maternal intravascular conductances associated with the maternal side and involved increases across the placenta (Table 3). These changes were as-
detectable differences in the partial conductances
Trophoblast, D
Maternal plasma, D
Conductances (ml/min/kPa) Non-smokers Smokers
Partial diffusive conductances of the placental oxygen pathway a
Table 3

\[\text{Maternal erythrocytes, } D_{\text{me}}: 63 \pm 0.46 \text{ ml/min/kPa/kg, smokers: 4.96} \pm 0.29 \text{ ml/min/kPa/kg.}\]

Despite this there were detectable differences in the partial conductances across the placenta (Table 3). These changes were asso-
ciated with the maternal side and involved increases in the maternal intravascular conductances \(D_{\text{mat}}\) and \(D_{\text{mph}}\) and amounted to 11 and 25%, respectively. In rela-
tive terms, the principal resistances to oxygen dif-
fusion (estimated as reciprocal partial conductances) across the placenta were provided by the trophoblast (76% of total resistance) and the stroma (14%).

The transfer of oxygen across the placenta will depend upon the partial pressures of oxygen in maternal and fetal plasma as well as the diffusive resistance of the placenta to oxygen transfer. The consistency of the total diffusive capacity between smokers and non-smokers could indicate that if there is a reduction in the oxygen transfer across the pla-
centa associated with smoking then it must arise from a reduction of the partial pressures of this gas in the maternal and fetal blood streams.

It has been known for some time from haematologi-
cal measurements that the fetus in pregnancies associ-
ated with maternal smoking is chronically hypoxic [5].

Indicators of this condition include an elevated mater-
nal haematocrit (a increase in the relative volume occu-
pied by the red corpuscles in the blood), and increases in total maternal haemoglobin, carboxyhaemoglobin, and levels of erythropoietin (a hormone that stimulates the production of haemoglobin). Fetal haemoglobin levels are also elevated by maternal smoking.

The present results are in agreement with these changes. In this study haematocrits for maternal blood were significantly higher in mothers who smoked (non-smokers: 36.4 \pm 1.07 (\(N = 30\)), smokers: 38.9 \pm 0.579 (\(N = 55\)) and this was reflected in the haematocrit of fetal (umbilical cord) blood (non-smokers: 51.6 \pm 1.25 (\(N = 31\)), smokers: 54.6 \pm 0.657 (\(N = 57\)). Carbon monoxide induces hypoxia owing to its capacity to bind with haemoglobin thus reducing the capacity of the blood to deliver oxygen to the body tissues. Hypoxic stress may thus arise as the consequence of ingesting carbon monoxide in cigarette smoke and this in turn can induce red cell proliferation in both mother and fetus.

Although, the present data suggest that impaired oxygen transport may arise from hypoxia and an associ-
ated reduction in the partial pressures of oxygen in the maternal and fetal blood, care must be exercised in making this conclusion. The changes associated with smoking seen in this study are different to those associated with states of placental hypoxia arising from high-altitude pregnancies, maternal anaemia, and pre-eclampsia [4]. In these cases, there are in-
creases in capillary volume density and trophoblast prolifera-
tion. In the present case, it must therefore be recog-
ised that there may be multiple effects on the placenta arising from smoking, these being a con-
sequence of the range of different potential toxins all present in tobacco smoke. Thus although carbon monoxide may induce hypoxic stress, the effects of the polycyclic hydrocarbons and cadmium may pro-
duce changes not seen in purely hypoxic situations.

3.4. Placental uptake of alanine

As described in the Section 2.6, alanine uptake was determined using vesicles formed from microvillous border membranes extracted from the placental tissue. Protein yields from the extraction were 4.02 \pm 0.50% (\(N = 21\)) and placental ALP enrichment was 27.7 \pm 2.8 (\(N = 21\)). This was in agreement with previous work in this laboratory and indicated a pure extraction of the microvillous border membrane. Vessel sided-
ness was not tested. However, in previous work us-
ing the same protocol as employed here, the binding of ConA-FITC to specific receptors in the glycocalyx (outer) surface of the vesicles indicated the vesicles are all ‘right side out’ [14].

Uptakes measured at 22 °C rose rapidly with time over the first 2 min of incubation, the rate of increase then slowed reaching a steady maximum value (equi-
librium uptake) after 30 min. The increase in uptake
was approximately linear with respect to time for the first 2 min.

Equilibrium uptake was determined by adjusting the osmolality of the incubation medium by addition of mannitol and incubating the MBV for 30 min. The osmolality of the incubation media was varied over the range 300–700 m osmol/l. If all the alanine is taken up into the vesicle osmotic space (i.e. no binding to the microvillous border membrane), an inverse relationship between uptake and reciprocal osmolality would be expected with zero uptake at infinite osmolality. This relationship was observed (correlation coefficient: 0.990 with 3 d.f.) and extrapolation of the line of best fit demonstrated that the uptake did not differ significantly from zero at infinite osmolality. All the alanine taken up by the vesicles therefore entered the osmotic space.

Linear phase uptakes were measured after 1 min in the presence and absence of sodium and in concentrations of alanine. Vesicles were suspended in incubation medium containing either Na$^+$ or K$^+$. Sodium-dependent uptake was determined by subtracting uptakes in the K$^+$ medium from the corresponding uptakes determined in the Na$^+$ medium. As demonstrated in Fig. 1, sodium-dependent uptakes were significantly greater for smokers compared to non-smokers ($P < 0.002$). In contrast there was no significant difference between smokers and non-smokers in relation to sodium-independent uptake (Fig. 2). Linear phase uptakes were related to alanine concentration using the Michaelis–Menten equation:

$$U = \frac{U_{\text{max}} [\text{Ala}]}{(K_m + [\text{Ala}])}$$

where $U$ is the linear phase uptake, $U_{\text{max}}$ the maximum uptake, and $K_m$ the Michaelis constant. The values of $U_{\text{max}}$ and $K_m$ derived from the curves shown in Fig. 2 are shown in Table 4. This result is in agreement with Sastry et al. [19] who also observed increased values of $U_{\text{max}}$ and $K_m$ associated with the uptake of $\alpha$-aminoisobutyric acid (AIB) by villous fragments of placenta derived from smoking and non-smoking mothers. If the supply of amino acids to the placenta is restricted (by changes in the maternal blood supply, for example) then an enhancement of amino acid transporter activity would act to compensate for this restriction. The present findings therefore may indicate an adaptive change to compensate for a deficit in the supply of amino acids associated with smoking. The present findings however contrast with Godfrey et al. [6] who did not observe any significant changes in sodium-dependent uptake of methylaminosobutyric acid (McAIB) between non-smokers and smokers, and a significant reduction in uptake by smokers of sodium-independent McAIB. Further work will be necessary to resolve this issue.

![Fig. 1. Sodium-dependent alanine uptake. Means ± S.E.M. shown for 27 smokers and 21 non-smokers; (●) smokers; (□) non-smokers.](image)
Table 4
Michaelis–Menten constants for alanine uptake

<table>
<thead>
<tr>
<th>Smoking status</th>
<th></th>
<th>Na-dependent uptakes</th>
<th></th>
<th>Na-independent uptakes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>$J_{\text{max}}$ (nmol/mg/min)</td>
<td>$K_m$ (mM)</td>
<td>$J_{\text{max}}$ (nmol/mg/min)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>21</td>
<td>1.17 ± 0.13</td>
<td>3.10 ± 0.60</td>
<td>2.22 ± 0.29</td>
</tr>
<tr>
<td>Smokers</td>
<td>27</td>
<td>2.51 ± 0.10</td>
<td>4.28 ± 0.26</td>
<td>2.23 ± 0.11</td>
</tr>
</tbody>
</table>

4. Conclusions

Maternal smoking habit has a significant effect upon the membranes of the placenta. The induction of EROD activity and the presence of cadmium in the placental tissues indicates components of ingested tobacco smoke reach the placenta. A significant decrease in fetal capillary volume was associated with smoking and it is possible this was a consequence of a change in the diameters of the capillaries rather than a change in their length. An increase in the diffusion distance across the trophoblast was observed. However, this was insufficient to cause a change in the partial oxygen diffusive capacity of this membrane. The total oxygen diffusive conductance of the placenta was not affected by smoking, although changes in maternal and fetal blood hematocrits and associated changes in red blood cell conductances indicated the presence of hypoxic stress in smokers. This effect might in turn be a consequence of carbon monoxide ingested in tobacco smoke. However, the changes in placental structure were different from those observed in other types of chronic hypoxia and this suggests that components other than carbon monoxide might have effect on membrane structure and function. Studies on vesicles formed from the microvillous border of the trophoblast indicated that the sodium-dependent uptake of alanine was greater in tissue extracted from smokers compared to that from non-smokers. It is possible that this represents a compensatory change in placental function in response to a more adverse milieu arising from the effects of smoking.

Acknowledgements

We are grateful to the midwifery staff at Aberdeen Maternity Hospital and, for grant support, to The Tobacco Products Research Trust.
References


