Biochemical Pharmacology, Vol. 16, pp. 497–502. Pergamon Press Ltd. 1967. Printed in Great Britain

SOME ASPECTS OF PLACENTAL CARBOHYDRATE METABOLISM IN THE RAT

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(*Received* 29 *May* 1966; *accepted* 20 *October* 1966)

Abstract—Lactic acid production, glucose uptake and glycogen utilization were measured in term rat placentae *in vitro* in aerobic and anaerobic conditions.

The addition of adrenaline to the incubation media had no influence on these parameters. The findings contrast with the results of similar studies in human placentae and in other tissues of the rat. It is suggested that the difference in response between rat and human placentae casts doubts on the validity of using rodents for the assessment of potential drug effects in pregnant women.

INTRODUCTION

PLACENTAL glycogen concentration in the rat reaches a peak at about the 16th day of gestation and declines thereafter,[1,2] whereas in man, placental glycogen decreases steadily from 8 weeks to term.[3] In view of the apparent paucity of comparative data on placental intermediary metabolism in the two species, and the increasing use of rats for the assessment of drugs prior to their administration in pregnant women, a study was undertaken of some aspects of placental carbohydrate metabolism in the rat comparable to previous studies in human placentae.[4–6]

METHODS

Fed pregnant Sprague–Dawley rats were used. At 20 days gestation (estimated to within 12 hr from the time of insemination), the rats were stunned and immediately decapitated. The uterus was taken out and the placentae, (generally 8–12 per rat) trimmed of cord and membranes, were rinsed in ice-cold 0·9% saline and drained on hardened filter paper. Slices of placental tissue, approximately 0·5 mm thick, were cut with a Stadie–Riggs microtome, the maternal surface of each placenta having been discarded.

Random slices of tissue, weighing about 100 mg, were incubated for 1 hr at 37° in 1 ml of either Krebs–Ringer bicarbonate buffer pH 7·4[7] or modified Krebs–Ringer phosphate buffer pH 7·4[8]. The incubation flasks were gassed with 95% O_2/5% CO_2 or 95% N_2/5% CO_2 for the experiments using bicarbonate buffer and with 100% O_2 for those with phosphate buffer. In different experiments (as indicated in the results) additions to the incubation media, were, glucose at a concentration of 11·1 mM and/or adrenaline (synthetic (1) adrenaline bitartrate (B.D.H.)) in a concentration of 10 or 40 μg/ml media.

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Lactic acid production was determined by measuring the amount of lactic acid present in the incubation media at the end of incubation by an enzymic method.[9] Placental glycogen content, before and after incubation was estimated by the anthrone method[10] after digestion of the tissue in hot KOH and precipitation of the glycogen by alcohol. Glucose uptake was determined by measuring the concentration of glucose in the incubation media before and after incubation using a glucose oxidase method.[11] The rates of lactic acid production, glucose uptake and glycogen utilization were calculated as described previously in studies of human placental metabolism,[4, 5] glycogen content being calculated in terms of glucose (μM/g wet weight of tissue) and its rate of disappearance during incubation being estimated from the mean content of the tissue before and after incubation and expressed as μM/g wet weight tissue /hr).

RESULTS

The results, expressed in μmole/g wet weight tissue $\pm$ standard error of the mean, are the mean values derived from the total number of flasks (indicated throughout in parentheses) which were incubated in the same conditions. In each experiment groups of 4–5 flasks containing tissue slices from the same rat were incubated in different conditions.

The mean aerobic placental lactic acid production in bicarbonate buffer with added glucose was 34·34, $\pm$ 0·94 (39) μM/g wet weight/hr. In the absence of added glucose substrate, placental lactic acid production was much less and averaged only 15·64 $\pm$ 0·49 (38) μM/g wet weight/hr. In anaerobic conditions, the corresponding values were 35·98 $\pm$ 1·79 (10) and 18·11 $\pm$ 0·84 (10) μM/g wet weight/hr; these means are not significantly different from the respective aerobic value. In phosphate buffer containing glucose, placental lactic acid production averaged 29·42 $\pm$ 1·54 (8) μM/g wet weight/hr.

Glucose uptake of rat placentae incubated in bicarbonate buffer averaged 17·87 $\pm$ 0·69 (31) μM/g wet weight/hr in aerobic conditions and 16·67 $\pm$ 1·25 (10) μM/g wet weight/hr in anaerobic conditions. These values are not significantly different. Glucose uptake in phosphate buffer was 13·24 $\pm$ 1·19 (4) μM/g wet weight/hr.

Mean glycogen content of rat placenta was initially 7·62 $\pm$ 0·61 (29) μM/g wet weight. After incubation in bicarbonate buffer, mean glycogen content fell markedly indicating a net utilization of placental glycogen during incubation. Values in different experimental conditions are shown in Table 1. The rate of glycogen breakdown

TABLE 1

	Glycogen content (μM/g wet weight)			
Added Substrate	Initial	O_2/CO_2	N_2/CO_2	P
Glucose (11·1 $\times$ 10^{-3}M)	5·30 $\pm$ 1·02 (4)	2·95 $\pm$ 0·14 (5)	2·20 $\pm$ 0·27 (4)	$<0·05$ $>0·02$
None	8·42 $\pm$ 0·59 (10)	2·85 $\pm$ 0·21 (9)	1·84 $\pm$ 0·06 (10)	$<0·001$

Glycogen content of rat placentae before and after incubation in bicarbonate buffer in the presence and absence of glucose in aerobic and anaerobic conditions. Values given are mean values $\pm$ SEM with the number of observations in parentheses. P denotes the level of significance for the difference in the glycogen content of the tissue after incubation in aerobic and anaerobic conditions.

(calculated from the initial tissue glycogen content and that present at the end of incubation) was greater in placentae incubated anaerobically than aerobically, irrespective of the presence or absence of glucose in the incubation medium.

The addition of adrenaline to the incubation media produced no significant change in either lactic acid production or glucose uptake (Table 2). The rate of glycogen

TABLE 2

Buffer	Added substrate (Glucose ($11{\cdot}1 \times 13^{-3}$M)	Control	Lactic acid production (μM/g wet weight/hr) +Adrenaline (10 μg/ml)	+Adrenaline (40 μg/ml)
Bicarbonate	+	$34{\cdot}14 \pm 1{\cdot}90$ (8)	$31{\cdot}87 \pm 2{\cdot}30$ (8)	—
Bicarbonate	+	$35{\cdot}81 \pm 2{\cdot}36$ (7)	—	$34{\cdot}82 \pm 3{\cdot}62$ (7)
Bicarbonate	−	$17{\cdot}35 \pm 1{\cdot}10$ (9)	$18{\cdot}72 \pm 1{\cdot}33$ (9)	—
Bicarbonate	−	$15{\cdot}45 \pm 1{\cdot}11$ (10)	—	$16{\cdot}12 \pm 1{\cdot}21$ (9)
Phosphate	+	$29{\cdot}42 \pm 1{\cdot}54$ (8)	$32{\cdot}07 \pm 1{\cdot}50$ (7)	—
			Glucose uptake (μM/g wet weight/hr)	
Bicarbonate	+	$16{\cdot}92 \pm 0{\cdot}81$ (4)	$15{\cdot}77 \pm 2{\cdot}37$ (4)	—
Bicarbonate	+	$17{\cdot}60 \pm 0{\cdot}88$ (7)	—	$17{\cdot}74 \pm 1{\cdot}20$ (7)
Phosphate	+	$13{\cdot}24 \pm 1{\cdot}19$ (4)	$15{\cdot}47 \pm 1{\cdot}26$ (4)	—

Influence of adrenaline on lactic acid production and glucose uptake of rat placentae incubated aerobically in bicarbonate or phosphate buffer with and without added glucose. Values given are mean values ± SEM with the number of estimates in parentheses.

breakdown in placentae incubated in bicarbonate buffer without glucose was 5·19 (20) μM/g wet weight per hr in the absence and 6·09 (19) μM/g wet weight/hr in the presence of adrenaline (40 μg/ml). These values are not significantly different.

DISCUSSION

Comparison of the present findings in rat placentae with those in human tissue,[4–6] incubated in similar experimental conditions, reveals a marked species difference. Thus, lactic acid production was very much greater in rat than in human placentae (Fig. 1)*, irrespective of whether the results were expressed in relation to wet or dry weight. Furthermore, whereas in the human placenta, the addition of adrenaline at a concentration of 40 μg/ml or incubation in anaerobic conditions increased lactate output by more than 50 per cent, there was no significant change in the rat (Fig. 2). The composition of the incubation medium has a profound influence on human placental lactate, lactic acid production being about 50 per cent higher in phosphate than in bicarbonate buffer;[4] in the rat, however, there was little difference in lactate output between the two media (Table 2). In other tissues of the rat, the *in vitro* effects of adrenaline vary with the composition of the incubating medium.[12]

Similarly glucose uptake was greater in rat than in human placentae and was not enhanced by the addition of adrenaline or by anaerobiosis (Fig. 3). Placental glycogen breakdown during incubation was much greater in rat than in man, (averaging 5·03 μM and 1·63 μM respectively), and in the rat was not influenced by the addition of adrenaline.

The marked differences in response between rat and human placentae cannot be

* The mean values for human placentae are derived from previously published data.[4–6]

attributed to variations in experimental procedure, for the conditions of incubation were the same in both series of experiments. The possibility that stunning or decapitation, as used in the rat but not in the human studies, was a contributory factor, was

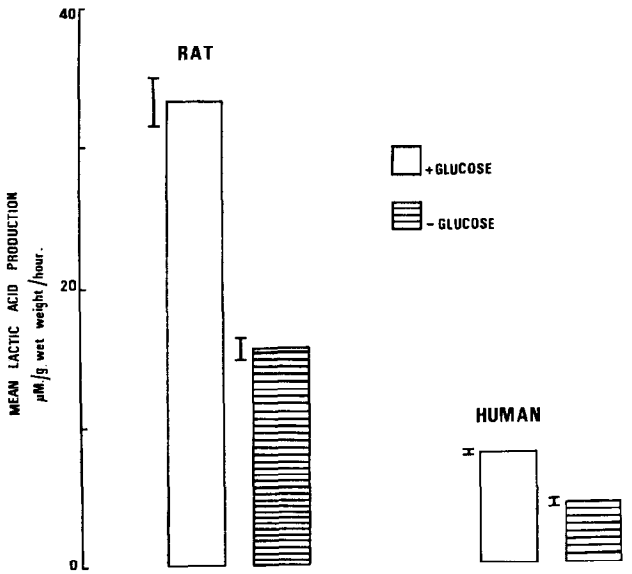

FIG. 1. Mean lactic acid production (μM/g wet weight/hr) of rat and human placentae incubated aerobically in bicarbonate buffer in the presence (□ open histograms) and absence (▤ hatched histograms) of glucose ($11{\cdot}1 \times 10^{-3}$M). The vertical lines represent the standard errors of the means.

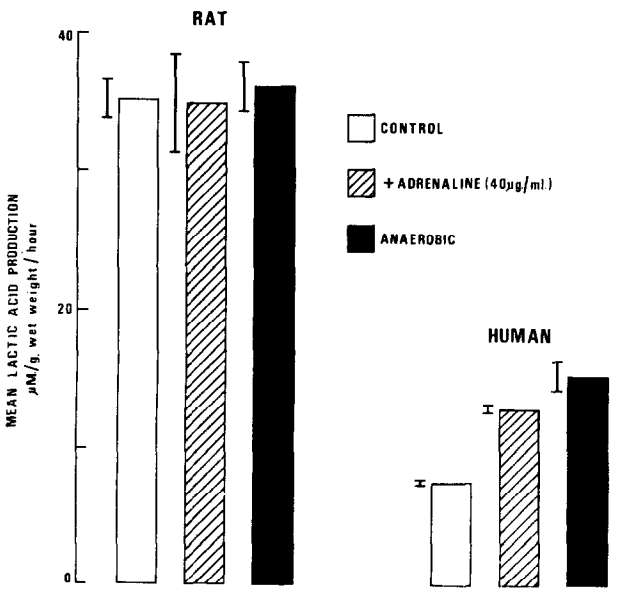

FIG. 2. Mean lactic production (μM/g wet weight/hr) of rat and human placentae incubated in bicarbonate buffer containing glucose ($11{\cdot}1 \times 10^{-3}$M). The open histograms (□) indicate placental lactic acid production in aerobic conditions, hatched histograms (▨) that in aerobic conditions in the presence of adrenaline (40 μg/ml) and closed histograms (■) that in anaerobic conditions. The vertical lines represent the standard errors of the means.

excluded by additional experiments. Parallel studies were carried out in sister rats: there was no difference in mean placental lactic acid production or glucose uptake and no significant effect of adrenaline on these parameters, in placentae obtained from rats under nembutal anaesthesia, after stunning and decapitation or from animals anaesthetised with nembutal and then decapitated.

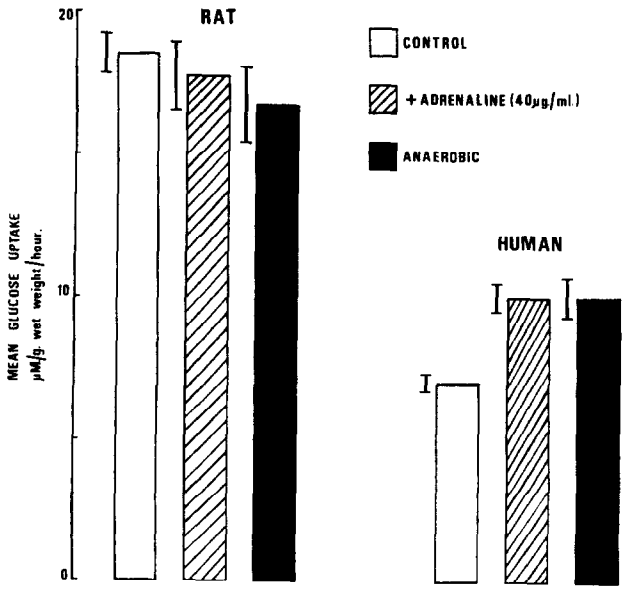

FIG. 3. Mean glucose uptake (μM/g wet weight/hr) of rat and human placentae incubated in bicarbonate buffer containing glucose ($11{\cdot}1 \times 10^{-3}$M). The open histograms (□) indicate placental glucose uptake in aerobic conditions, hatched histograms (▨) that in aerobic conditions in the presence of adrenaline (40 μg/ml) and closed histograms (■) that in anaerobic conditions. The vertical lines represent the standard error of the means.

It is difficult to provide a complete explanation for the very high rate of aerobic lactic acid production in rat placentae. The higher rates of glucose uptake and glycogen utilization in the rat could, theoretically, account for the difference in placental lactate production in the two species when the tissue was incubated with added glucose. However, the amount of glycogen disappearing in the absence of added substrate, cannot be equated with the excess lactate produced by the rat placenta. An alternative explanation is that some of the placental lactic acid in the rat is derived from compounds other than tissue glycogen or added glucose substrate, a possibility which requires further investigation of placental intermediary metabolism in rodents.

The fact that anaerobiosis caused no significant rise in lactic acid production or glucose uptake in the rat, contrasts markedly with the response in human placentae where lactic acid production is almost doubled, and glucose uptake is increased by about 30 per cent in anaerobic conditions.[6] The increase in glycogen breakdown during incubation in anaerobic conditions was, however, of the same order in the placentae of both species (20–30 per cent above the corresponding aerobic values). Hence, in anaerobic conditions, even if all the extra glycogen broken down were converted to lactic acid, only about 2 μM/g wet weight placental tissue per hour extra

lactate would be produced and in view of the very high basal level of placental lactate production in the rat, an increase of this order might not be statistically significant. A similar failure of anaerobiosis to increase lactate production and glucose uptake has been demonstrated in the rat retina, a tissue which also has an extremely high rate of aerobic glycolysis.[13]

A further possibility is that variations in the action of enzymes or co-enzymes of the glycolytic pathway might, as suggested by Dickens and Greville,[14] be responsible for the different rates of anaerobic glycolysis in rat and human placentae. It is, therefore, of interest that the concentrations of NAD and $NADH_2$ are lower in rat than in human placentae.[15]

The failure of adrenaline to enhance placental lactate production in the rat might, in part, be related to increased placental destruction of the amine, for though monoamine oxidase activity is, if anything, less in rat than in human placentae,[16] there is no information on placental catecholomethyl transferase activity in the two species. However, in rat placentae, the excessively high concentration of 160 μg/ml adrenaline had no effect on lactic acid production, whereas in human placentae, a concentration of only 0·625 μg/ml adrenaline significantly increased lactate production.[4] The possibility of altered tissue binding of adrenaline in rat placentae is also a matter for further investigation.

The rat is widely used for experimental purposes and, in the majority of organs, anatomical structure and physiological responses are surprisingly similar in rat and man. Hence the results of studies in rats have frequently been used in the analysis of physiological and pharmacological function in the corresponding human tissue. In the case of the placenta and foetal membranes, however, there are well-known anatomical differences and in view of the present evidence that there are also profound biochemical differences between the placentae of the two species, one may question the usefulness and even the validity of using rodents for assessing potential drug effects in pregnant women.

Acknowledgements—This work was supported by the Clinical Research Fund, Charing Cross Hospital, The Dan Mason Research Foundation and J. Sainsbury and Sons Ltd.

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