Hepatic glucokinase activity in animals fed glucose at birth.

HELENE LYALL1, DAVID TOSH2 and ANN BURCHELL3.

1. Dept. of Obstetrics and Gynaecology, Simpson Memorial Maternity Pavilion, Edinburgh Royal Infirmary, Edinburgh, 2. Department of Biochemistry 4 West, University of Bath, Bath BA2 7AY. 3. Dept. of Obstetrics and Gynaecology, Northwells Hospital, Dundee, DD1 9SY.

Introduction and rationale of the study

Recent work has suggested that non-insulin dependent diabetes and other conditions, e.g. ischaemic heart disease and hypertension, may have their origins in impaired growth and development during early fetal life and infancy. These diseases may be consequences of 'programming' where a stimulus or insult at a critical sensitive period of early life results in long term changes in physiology or metabolism. (1). Organs and systems mature during periods of rapid growth in fetal life and infancy, and there are critical time periods during which maturation must be achieved. Nutritional deprivation in early life can programme the size and DNA content of many organs and systems, those affected being dependent upon the exact time at which the insult occurs (2). Recent reports have demonstrated that early nutrition may play an important role in the later development of diabetes. Hales and Barker suggest that the same factors determine early growth as influence the β-cell mass in adulthood (3), and suggest that poor nutrition early in life may interact adversely with abundant nutrition later on, leading to impaired glucose-tolerance, and possibly type II diabetes (4). Other studies suggest that the autoimmune damage to islet cells responsible for type I (insulin-dependent) diabetes may also have a nutritional, although different trigger in early life (5).

The idea that events at a critical stage of development may influence the expression of glucokinase (GK) has yet to receive detailed attention. However, given the importance of this enzyme in blood glucose homeostasis, the possibility that maternal nutrition during pregnancy, nutrition in the perinatal period, or other as yet undefined factors may manifest themselves by changes in such key enzymes is worthy of attention. We have examined the ontogeny of rat and guinea-pig GK, and determined the effect of the administration of glucose in the immediate perinatal period on this developmental profile. The level of GK activity in the liver of the rat and other mammalian species has long been known to vary with the nutritional status of the animal, and moreover, studies have reported linkage of the MODY phenotype to the GK locus in a number of multigenerational pedigrees with the disease (6,7), providing a further impetus for this study.

Materials and Methods

Neonatal rats were used. Upon delivery of a litter of rats, the animals were individually weighed and sexed. The litter was separated into two groups matched for weight and sex. The test animals were injected intra-peritoneally with 0.5 ml 50% glucose and the control animals injected intra-peritoneally with 0.5 ml water before feeding by the mother. This volume of 50% glucose produced the most reproducible results in terms of achieving hyperglycaemia. The animals were returned to their mother and allowed to suckle normally. The liver was immediately removed and placed in sucrose-Hepes buffer. Microsomes were made as described (8). The experiment was repeated with neonatal guinea-pigs born at term. Guinea-pigs were also delivered prematurely by Caesarean section. The latter was performed when the pelvic bones exhibited a separation of 1/4 to 1 inch which was equivalent to 5 to 7 days before delivery in comparison with animals allowed to go to term. The animals were weighed and sexed, and maintained and tested. The test animals received 0.5 ml 50% glucose intra-peritoneally at 2 hourly intervals throughout the day in order to establish and maintain hyperglycaemia.

GK activity was determined by the method of Agius and Tosh (9).

Results

Figure 1 compares the developmental profile of GK in rat liver from birth to 172 days in control animals, and litter-mates which received perinatal glucose administration (PGA). As shown, GK does not appear until about 16 days after birth, thereafter, GK values steadily increase to adult values (figure 5.16A). Little effect of perinatal glucose administration was observed.

Figure 2 shows the ontogenetic profile of hepatic GK in the term guinea-pig. Initially GK levels are undetectable, then increase at approximately 30 days which coincides with weaning. The effect of PGA is difficult to determine due to the paucity of values. However, on the basis of the results collated and illustrated in Figure 1, there does not appear to be a premature induction of GK activity in response to PGA. However, there is a suggestion of a decrease in activity in enzyme animals in which had received PGA compared to control litter-mates.

Figure 3 shows an ontogenetic profile for GK in the preterm guinea-pig liver. Levels are shown to be low initially with an overshoot at approximately 30 days, a time which coincides with weaning. Thereafter, GK levels fall, subsequently maintaining a constant level up to 200 days. This situation is distinct to that observed in term guinea-pigs (compare Fig. 2). The effect of PGA on this ontogenetic profile is also shown. There appears to be a changing pattern of the effect of PGA as development proceeds. Initially from birth to approximately 100 days of age, there is a general decrease in hepatic GK activity in test animals compared to their control litter-mates. However, after 100 days of age, the overall trend appears to be that of an increase in GK activity in test animals compared to their control litter-mates.

Discussion

Using these experimental model systems, we wanted to determine whether it would be possible to alter in particular to decrease enzyme expression by the creation of a stimulus or insult in the perinatal period, in this case perinatal glucose administration. If this was so, would this altered expression persist in the long-term, thereby exerting an influence upon physiology and metabolism at a time far removed from the perinatal period? In addition to these questions, using the guinea-pig model, where enzyme ontogeny could be studied in the term and preterm situations in tandem, we wanted to determine the influence of premature alone upon subsequent enzyme expression.

The developmental profile of rat liver GK activity reported here is in good agreement with other studies (10-12). Glucokinase first appears in the liver of the rat two weeks after birth and its activity rapidly increases after weaning onto a high carbohydrate diet. Perinatal administration of glucose resulted in a change in GK activity in the rat (Fig.1).

The results obtained following PGA in the guinea-pig animal models both term and preterm are shown in Figs 2 and 3. Glucokinase ontogeny exhibited a different pattern in the term and preterm guinea-pig. In the term guinea-pig GK levels were initially low and increased at weaning reaching adult values which were thereafter largely maintained. In the preterm guinea-pig there was an initial over-shoot reaching values much higher than those seen in the term model. Thereafter, GK values declined and seemed to plateau at levels less than those seen in the term animals. Glucokinase activity exhibited a response to PGA with a general decrease initially followed after 100 days by an increase in activity observed in test animals compared to controls.

This work suggests that it is possible to alter GK activity by a stimulus or insult a sensitive period of development, in this case the perinatal administration of glucose. We have also shown that altered enzyme activity may persist at a time far beyond the perinatal period, and as the enzyme studied are of pivotal importance in carbohydrate metabolism, by inference such alterations have the potential to cause long-term changes in physiology and metabolism.

The term guinea-pig is particularly a model for term human infants, and indeed here perinatal glucose administration had a negligible effect on enzyme activity. This may be because as term infants are largely resistant to hypoglycaemia the latter and its attendant hormonal changes is less important as a trigger for enzyme development at birth.

The rat and preterm guinea-pig have been established as models for premature human infants. Some effect of perinatal glucose administration on GK in the preterm guinea-pig liver was observed, and it is interesting therefore to note that as models for premature infants, perinatal glucose administration may play a pivotal role in the subsequent development of enzyme activity in this high risk group.

References