EFFECT OF GLUCOCORTICOIDS ON INSULIN SENSITIVITY IN NEWBORN DOGS

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Summary: A 3-day treatment with 4 *mg/kg* Betnesol in anesthetized mongrel pups, 4-55 days of age effectively raised fasting plasma glucose concentration by 23±2.9%, which was higher than that observed in adult dogs similarly treated. Possibly this was due to the lack of effectiveness of insulin in the pups which is normally produced in response to hyperglycemia. The relative lack of effectiveness of insulin induced hypoglycemia in stimulating hepatic glucose production in the newborn pups was further demonstrated by the fact that insulin administration produced a hypoglycemia which lasted much longer than in adult dogs. On the other hand Betnesol treatment was found to be effective in restoring normoglycemia following administration of insulin in these pups presumably due to its gluconeogenic effect through the liver. In general this investigation points towards the lack of development of appropriate feedback mechanisms for glucose homeostasis in the newborn pups, and that it takes a period of about 3-4 weeks after birth to develop such mechanisms.

Key words: Betnesol insulin gluconeogenesis feedback mechanisms glucose homeostasis

INTRODUCTION

Nixon (10) reported an insensitivity to insulin in the calf foetus at a stage of gestation at which glucose is able to mobilize insulin from the pancreas. Our results of an earlier study (7) showed that insensitivity to insulin persisted after birth. Administration of relatively high doses of bovine insulin to pups revealed a marked insensitivity to the injected insulin during the first four hours of life. A sluggish hypoglycemic response appeared in pups between the age of 4-16 hours. In pups older than 16 hours a well-developed hypoglycemic response to insulin was observed though the recovery to preinjection plasma glucose level was absent or much delayed.

The slow recovery to normoglycemia could be due to a lack of a feedback mechanism between the plasma concentration and hepatic rate of production of glucose (7). This failure of the liver of the newborn to increase glucose production in response to hypoglycemia is not well-understood. It is possible that the liver in the newborn lacks the enzymic machinery to increase glucose production. If this is so then administration of glucocorticoids which are known to increase activity of some enzymes involved in gluconeogenesis (4, 5, 13, 14) would be effective in increasing hepatic glucose production in the newborn. Therefore, the present study was carried out to see if treatment with glucocorticoids will alter the response to injected insulin in the newborn.

MATERIALS AND METHODS

The experiments were carried out on 14 mongrel pups of both sexes from 3 litters and 3 adult female dogs. The ages of the pups ranged from 4 to 55 days and their weights ranged between 220 *gm* to 1 *kg*. The animals were anesthetized with an intraperitoneal injection of 30 *mg/kg* Nembutal after a fast of 4 to 6 hrs. A polythene cannula was inserted into left common carotid

artery in smaller animals and into left femoral artery of bigger pups. Serial blood samples were drawn through this cannula. Rectal temperature was monitored and kept constant at 37±0.5°C with the help of an automatic heating pad.

Three adult dogs, the mothers of the pups were also experimented upon, 6 weeks post-partum. These three animals were fasted for 16 hours prior to the experiment. They were anesthetized by injecting 30 *mg/kg* of Nembutal intravenously. A polythene cannula was inserted into the inferior vena cava via the saphenous vein for drawing of the blood samples.

Experimental design : Pups of the same age and litter were paired. One pup in each pair received 4 *mg/kg* per day of Betnesol (Betamethasone sodium phosphate) intramuscularly for 3 days. The other pup served as control.

On the fourth day the control as well as Betnesol treated pups were experimented upon. Two control blood samples were withdrawn at intervals of 10 minutes and Bovine insulin 0.12 *U/kg* was injected intra-arterially in both the control as well as the glucocorticoid treated animals. Serial blood samples were withdrawn at 5, 15, 30, 45, 60, 90 and 120 minutes after the injection of insulin. The amount of blood withdrawn in each sample did not exceed 0.15 to 0.2 *ml* and an appropriate amount of 0.9 per cent NaCl solution was injected after the withdrawal of each sample. The blood samples were withdrawn by using a dry tuberculin syringe and collected in small tubes containing dry heparin. The blood glucose estimations were done by the micro-analytical method described by Sharma *et al.* (6). Following the experiment the animals were sacrificed by an overdose of Nembutal.

In 3 adult animals (mothers of the pups) the insulin sensitivity tests were done twice by injecting 0.12 *U/kg* of bovine insulin each, once before and once after 3-day treatment with Betnesol. Thus each animal acted as its own control. Statistical analysis was done by standard techniques. All errors cited in the text are standard errors for the mean.

RESULTS

Table I shows the ages, weights and fasting plasma glucose concentrations of the experimental animals. The fasting plasma glucose concentrations in the control pups and their matched pairs which received Betnesol for 3 days were 87±3.6 and 110±4.9 *mg*/100 *ml* respectively. The average increase being 23±2.9 *mg*/100 *ml*. This difference in plasma glucose level in the treated and control pups was statistically significant ($t=3.782$, $p=0.01$). Although, largest difference was observed in the youngest pair of pups (4-day old) but the correlation between the differences in plasma glucose concentration within the pair and their age was not significant. Betnesol treatment in the adult mothers of the pups produced an average difference of 6.3 *mg*/100 *ml* in their plasma glucose concentrations, which was much less than that observed in the pups.

Sensitivity to injected insulin :

Insulin sensitivity tests were performed on 7 pairs of pups aged 4, 7, 8, 14, 21, 33 and 55 days each. A clearcut hypoglycemic response was observed in all the pups. However in the youngest 4

TABLE I: Effect of Betnesol treatment on weight and plasma glucose concentration in pups.

Litter No.	*Age (days)*	*Body weight (kg)*		*Fasting plasma glucose mg/100 ml*		
		—Betnesol	*+Betnesol*	*—Betnesol*	*+Betnesol*	*Difference*
1	4	0.22	0.25	50	96	+46
1	7	0.38	0.44	104	136	+32
2	8	0.26	0.32	91	94	+ 3
3	14	0.40	0.54	63	84	+21
2	21	0.72	0.86	94	132	+38
1	33	0.64	0.76	125	158	+33
3	55	0.90	1.00	84	72	—12
Mean ± S E M				87±3.6	110±4.9	23±2.9 (t=3.782, p=<.01)
1	Mother	10.0	9.8	63	77	+ 14
2	Mother	8.5	8.2	95	100	+ 5
3	Mother	8.8	8.8	113	113	0
Mean				90.3	96.6	+6.3

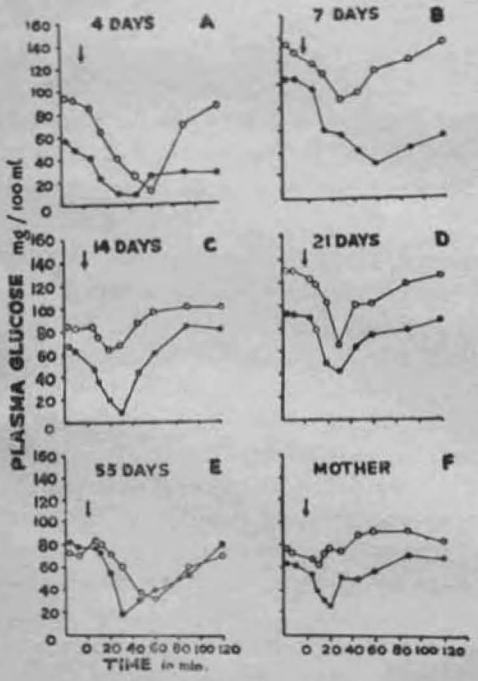

Fig. 1 : The time course of plasma glucose concentrations in control (●——●) and Betnesol treated (○——○) pups following insulin injection. The arrow marks the intravenous administration of insulin (0.12 *U/kg*). The ages of the pups are shown on the graphs.

days old control pup the lowest plasma glucose concentration reached at 45 minutes after insulin injection and the recovery to preinjection plasma glucose level was incomplete. Whereas in the Betnesol treated pup of this pair the lowest plasma glucose value was observed at 60 minutes and there was 87% recovery in two hours (Fig. 1A). A similar pattern of hypoglycemic response to injected insulin was observed in 7 days old pair of pups (Fig. 1B). The recovery to preinjection plasma glucose level was virtually absent in the control pup, whereas the treated pup showed almost complete recovery (97%). Fig. 1C shows the course of hypoglycemic response to insulin in a 14 days old control pup and the Betnesol treated pup of the same age. In the control pup the plasma glucose fell steeply from a control value of 63 *mg*/100 *ml* to 7 *mg*/100 *ml*, 30 minutes after the administration of insulin and the recovery was 100 per cent. In the treated pup following insulin injection, the plasma glucose fell from a control value of 84 *mg*/100 *ml* in 20 minutes and it recovered completely in 45 minutes. In pups older than 2 weeks, the hypoglycemic response to injected insulin was very similar in the control as well as Betnesol treated pups. Fig. 1D and E show insulin sensitivity tested on 21-day and 55-day old pairs of pups respectively. The plasma glucose concentration decreased sharply following administration of insulin. The lowest concentration was achieved in 30 minutes and complete recovery to preinjection level occured in about 90-120 min, in control as well as Betnesol treated pups. These responses are not significantly different from those observed in the adult animal, the mother of the pups (Fig. 1F), except that the hypoglycemic responses in the pups are generally sluggish. The initial fall of plasma glucose concentration in pups is less steep. The lowest value is reached later in the pups and the recovery is also delayed as compared to the adult animal.

DISCUSSION

The plasma glucose concentration in the pups was elevated by Betnesol treatment. This is essentially due to gluconeogenesis by the liver (4, 5, 8, 14). The average increase of 23 ± 2.9 per cent was higher and more variable than that observed in adult dogs (mothers of the pups) in which the average increase was 6.3 per cent. Issekutz and Allen (8), Ninomiya *et al.* (9) and Cowan *et al.* (2) also found lesser increase in plasma glucose concentration by glucocorticoid treatment in adult animals.

In our earlier study (2) the rate of glucose production was found to be elevated by 180.5% in adult dogs following 3 day treatment with methylprednisolone. However, similarly treated pups between the ages of 4-47 days showed an average increase of only 34.3% which would indicate that glucocorticoid treatment is about 5 times more effective in increasing the hepatic glucose production in adult animals than it is in newborn. Although, the glucose production in mature animal is elevated to a greater extent, yet their plasma glucose concentration is not raised as much as it is in the newborn following glucocorticoid treatment. The likely reason for this is that in the adult the enhanced glucose production leads to hyperglycemia which in turn causes release of extra insulin (1, 11). Thus hyperglycemic effect of glucocorticoids is compensated by their insulinotropic effect and normoglycemia is restored thereby. In the newborn also the hyperglycemia produced by glucocorticoids releases large amount of insulin but the pups are relatively much less sensitive to the effects of insulin (12). Therefore, their fasting plasma glucose concentration remains high. Thus, it appears that newborn dogs are not able to maintain glucose homeostasis as well as mature animals.

Sensitivity to injected insulin :

A study of hypoglycemic response obtained by administration of insulin in pups showed that the response is generally sluggish as compared to that obtained in the adult. This is consistent with our earlier findings in which newborn dogs were found to be insensitive to injected as well as to their own endogenous insulin (7). This decreased sensitivity might be of some biological value to the newborn in which a number of environmental and metabolic factors tend to favour the occurrence of hypoglycemia. However, the reason for this is not known.

Further a comparison of hypoglycemic response following insulin injection in 4 days old control and Betnesol treated pups indicated that the return of plasma glucose level to preinjection level was much delayed and incomlete in the control pups. Whereas in the Betnesol treated pups the recovery to normoglycemia was quicker and almost complete (87% and 97% respectively). This slow return of plasma glucose concentration to preinjection level in the control pups could possibly be due to the lack of feedback mechanism operating between the plasma concentration of glucose and its hepatic production in the newborn (7). Normally in adults the glucose production increases with hypoglycemia and decreases with hyperglycemia to restore normoglycemia (3). However, in the newborn hypoglycemia fails to trigger an increased glucose production. It appears that treatment with Betnesol restores the ability of liver to increase glucose production in response to hypoglycemia. It seems likely that glucocorticoid treatment does stimulate gluconeogenesis in the newborn also. This stimulation of gluconeogenesis seems to be age dependent. Thus Betnesol treatment 4 days old pup showed lesser recovery of plasma glucose to preinjection level as compared to similarly treated 7 days old pup in response to insulin injection. In pups aged 14 days or more the gluconeogenesis seems to be well-developed and therefore the hypoglycemic response to insulin administration is not much different in control and Betnesol treated pups. The recovery of plasma glucose to preinjection level is complete in all pups older than 2 weeks. A study of the hypoglycemic response in Betnesol treated older pups and mothers of the pups showed that injected insulin produces lesser hypoglycemia in them as compared to their matched control animals. This could be due to excessive levels of glucocorticoids which are known to exert an antiinsulin effect.

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