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Presence of Pancreatic Glucagon in the Portal Plasma of Human Neonates. Differences in the Insulin and Glucagon Responses to Glucose Between Normal Infants and Infants from Diabetic Mothers

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Summary. Human neonates have been studied during the first hours of life. Blood glucose, portal plasma insulin and glucagon have been determined both at regular intervals up to 24 h after birth and during an intravenous glucose load performed at the 24th h. A material presenting the immunological characteristics of pancreatic glucagon has been found in the portal plasma of both normal infants and infants from diabetic mothers (IDM). The intravenous glucose load did not suppress plasma glucagon in the normal neonates nor in the IDM. Higher portal plasma glucagon values were observed in the late phase of the intravenous glucose load in normal neonates compared to IDM. Portal plasma insulin has been found higher in IDM both at the 24th h of life and during the early phase of the intravenous glucose tolerance test. The hypothesis is put forward that the behaviour difference in glucagon secretion might be a consequence of the relative hyperinsulinism of IDM with insulin facilitating the entry of glucose into the α cell thus permitting a more effective glucagon suppression.

Présence de glucagon pancréatique dans le plasma portal de nouveau-nés. Différences de réponses de l'insuline et du glucagon au glucose entre les enfants normaux et les enfants de mères diabétiques.

Résumé. Les auteurs ont étudié des nouveau-nés humains pendant les premières heures de la vie. La glycémie, les taux d'insuline et de glucagon dans le plasma portal ont été dosés à intervalles réguliers jusqu'à la 24ème heure après la naissance, de même què au cours d'une surcharge glucosée intraveineuse pratiquée à la 24ème heure. — Un matériel présentant les caractéristiques immunologiques du glucagon pancréatique a été mis en évidence dans le plasma portal des nouveau-nés normaux et de mère diabétique. La surcharge glucosée intraveineuse ne réduit pas le taux de glucagon plasmatique chez le nouveau-né normal ni chez l'enfant de mère diabétique. — Dans la phase tardive de la surcharge glucosée intraveineuse, les valeurs de la glucagonémie portale sont plus élevées chez l'enfant normal que chez le nouveau-né de mère diabétique. L'insulinémie portale est plus élevée chez le nouveau-né de mère diabétique à la 24ème heure de la vie et à la phase initiale de la surcharge glucosée. — L'hypothèse est proposée que la différence de comportement du glucagon pourrait résulter de l'hyperinsulinisme relatif de l'enfant de mère diabétique, l'insuline favorisant la pénétration de glucose dans la cellule α et permettant, par ce mécanisme, une suppression plus efficace de la sécrétion de glucagon.

Anwesenheit von Pancreasglucagon im portalen Plasma von menschlichen Neugeborenen. Verschiedenartigkeiten in der Insulin- und Glucagonantwort nach Glucose zwischen normalen Kindern und Kindern diabetischer Mütter

Zusammenfassung. Menschliche Neugeborene wurden während der ersten Lebensstunden untersucht. Blutglucose, portales Plasmainsulin und Glucagon wurden sowohl in regulären Abständen bis zu 24 Std nach der Geburt als auch während einer intravenösen Glucosebelastung in der 24. Std untersucht. — Eine Substanz mit den immunologischen Charakteristika von Pancreasglucagon wurde im portalen Plasma sowohl von normalen Kindern als auch von Kindern diabetischer Mütter gefunden. Die intravenöse Glucosebelastung hat weder bei den normalen Neugeborenen noch bei den Kindern diabetischer Mütter das Plasmaglucagon unterdrückt. Im Vergleich zu den Kindern diabetischer Mütter wurden bei den normalen Neugeborenen in der späten Phase der intravenösen Glucosebelastung höhere Plasmaglucagonwerte beobachtet. Portales Plasmainsulin war bei den Kindern diabetischer Mütter sowohl nach 24 Std als auch während der ersten Phase des intravenösen Glucosetoleranztests erhöht gefunden worden. — Es wird die Hypothese vorgeschlagen, daß das Verhalten der Unterschiede in der Glucagonsekretion möglicherweise eine Folge des relativen Hyperinsulinismus der Kinder diabetischer Mütter sei, welcher der Glucose den Eintritt in die Zelle durch Insulin erleichtert und so eine effektvollere Glucagonverminderung erlaubt.

Key words: hypoglycemia, glucagon, neonate, intravenous glucose, hyperinsulinism, diabetes, pancreatic α-cell, diabetic mother, portal plasma.

Introduction

Studies on the physiology of glucagon have been hampered by major difficulties in the development of a sensitive and specific assay procedure (see Luyckx and Lefebvre, 1970). This undoubtedly explains the total lack of data on the regulation of glucagon secretion in the newborn. This paper provides preliminary results indicating, for the first time, that circulating pancreatic glucagon is present in the portal plasma of the human neonate. It also reveals a distinct difference in the glucagon response to an intraportal glucose load, between normal infants and infants from diabetic mothers.

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Material and Methods

Thirteen normal neonates and five infants from diabetic mothers (IDM) were studied during the first 24 h after birth. The principal characteristics of the children and mothers are summarized in Table 1.

In all infants, a catheter was introduced into the portal vein, via the umbilical vein, in order to perform blood sampling and glucose infusion. In every case, the location of the catheter in the portal vein was verified radiographically. Throughout the 24 h study period, no oral feeding was permitted. In 7 of the normal infants and in 4 of the IDM, portal blood glucose and portal plasma insulin and glucagon levels were determined 6, 12, 18 and 24 h after birth. In addition, in 7 of the normal infants and in all the IDM an intraportal glucose load (1 g/kg BW) was administered at the 24th hour after birth. Portal blood glucose and portal plasma insulin and glucagon

Table 1

Infant	Birth weight	Mother	Delivery
T. M. ♂	3150	normal	vaginal at term
Bi. G. ♂	4050	normal	vaginal at term
C. M. ♂	3300	moderate toxemia	vaginal at term
Br. G. ♂	3000	normal	vaginal at term
S. L. ♀	4330	normal	vaginal at term
M. B. ♀	3400	moderate toxemia	vaginal at term
B. L. ♀	3450	normal	vaginal at term
B. F. ♂	3500	normal	vaginal at term
C. G. ♀	2950	normal	Caesarian section at term
P. S. ♀	3110	normal	vaginal at term
R. M. ♂	3800	normal	vaginal at term
T. A. ♀	2450	normal	vaginal at term
R. M. ♂	3000	normal	vaginal at term
G. A. ♀	3450	mild diabetes treated by diet alone	vaginal at term
R. S. ♀	4700	gestational diabetes treated by diet alone	vaginal at term
L. G. ♀	4500	mild diabetes treated by diet alone	vaginal at term
M. V. ♀	3700	insulin treated diabetes	vaginal at term
V. C. ♂	4350	gestational diabetes treated by diet alone	Caesarian section at term

levels were assayed before and at several intervals (see Tables) after the glucose load. Blood glucose was determined enzymatically (Huggett and Nixon, 1957) plasma insulin by radioimmunoassay (Hales and Randle, 1963) and plasma glucagon by an immunoassay using rabbit antiglucagon serum (final dilution 1/1800), 15 pg/tube of 131 I-labelled glucagon, pork glucagon as a standard and a charcoal separation of antibody-bound and free hormone (Leclercq-Meyer *et al.*, 1970). The antiserum used showed a weaker affinity for a total gut extract than for pancreatic glucagon, which classes it as "partially discriminant" (Heding, 1972).

The sensitivity and reproducibility of standard-curves for this type of assay are illustrated in Fig. 1. Within one assay series, and using duplicate determinations, 12.5 pg of glucagon per tube (125 pg/ml) caused a significant decrease (8.2%) of the percentage of labelled glucagon bound to the antibody ($p < 0.01$). The accuracy of the method for values between 0 and 500 pg/ml is 6.7 $\pm$1.5 (S.E.M.)% (duplicates on 20 random determinations).

Results

1. The assay system used in this study demonstrated the presence of a material reacting with glucagon antibodies in the portal plasma of both normal neonates and IDM. The dilution curve of this immunoreactive material was perfectly parallel to that of the crystallized pancreatic glucagon used as a standard (Fig. 2), which strongly suggests that the material is pancreatic in origin. The portal plasma glucagon values obtained during the first 24 h of life are summarized in Table 2. As shown in this table, the values in normal

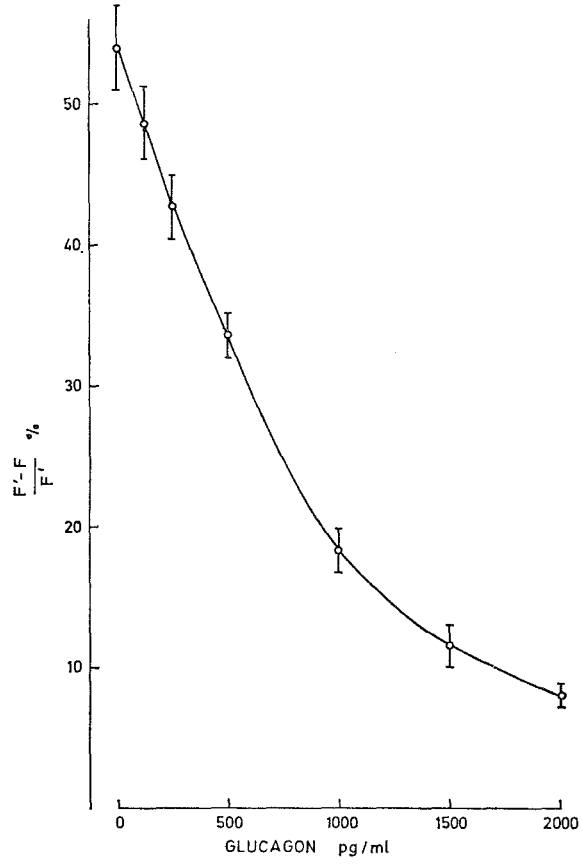

Fig. 1. Mean of 8 successive standard curves performed during a 4 month period. Comparison of paired values for zero and 125 pg/ml (12.5 pg/tube) reveals a highly significant difference $p < 0.001$. F′ corresponds to the amount of radioactivity bound to charcoal in the absence of antiserum and F to the amount of radioactivity bound to charcoal in the presence of antiserum and various concentrations of crystalline glucagon or plasma

infants ranged from 290 to 4800 pg/ml and, in every case but one a distinct rise was observed over the first few determinations. In 2 of the 4 IDM, much higher figures were obtained. The means were 1460 $\pm$ 325 pg/ml (S.E.M.) for the normal infants and 3750 $\pm$ 1750 pg/ml (S.E.M.) for the IDM. This difference is not, however statistically significant ($p < 0.1$). In contrast, portal plasma insulin is significantly higher

at the 24th h in IDM: 74.5 ± 11.7 ($n=6$) *versus* 37.2 ± 2.4 ($n=4$) μU/ml ($p<0.02$).

2. The glucose assimilation constant (K) after intravenous glucose load was calculated according to Conard (1955). The K value was significantly higher (1.86 ± 0.27) in the IDM than in the normal neonates (1.19 ± 0.13; $p<0.05$) (Table 3).

4. Data on portal plasma glucagon, before and after intraportal glucose are given in Table 5. In the normal newborn, portal plasma glucagon concentrations changed only slightly and insignificantly during the first 30 min of the test. This was also true for IDM. Subsequently, an increase to 317% at the 60th min and 834% at the 90th min was noted in normal infants.

Table 2. *Portal vein plasma glucagon and blood glucose values in the newborn*

	Normal mother					Diabetic mother			
Hour	06	12	18	24		06	12	18	24
T. M.	920	1050		850	G. A.		520	510	620
	34	30		38			88	100	88
	50	*52*		*60*			*48*	*48*	*66*
Bi. G.	540		710	610	R. S.	990	380	590	530
	43		75	28		46	27	52	50
	71		*114*	*60*		*40*	*10*	*10*	*60*
C. M.	290	600		560	L. G.	>19550	1980	2610	2020
	34	38		30		130	120	115	100
	30	*91*		*76*		*25*	*48*	*40*	*61*
Br. G.		410	510	320	M. V.	5070	19960	2470	2170
		33	28	29		140	115	77	60
		74	*72*	*60*		*66*	*28*	*10*	*32*
S. L.	2060	3040	960	200					
	80	60	54	46	For each subject, the first figure corresponds to glucagon concentrations (pg/ml of portal plasma), the second to portal plasma insulin (μU/ml) and the third to portal blood glucose concentrations (mg %)				
	86	*76*	*86*	*81*					
M. B.		570		1250					
		50		42					
		86		*99*					
B. L.		4800	4800	4080					
		42	36	—					
		65	*70*	*70*					

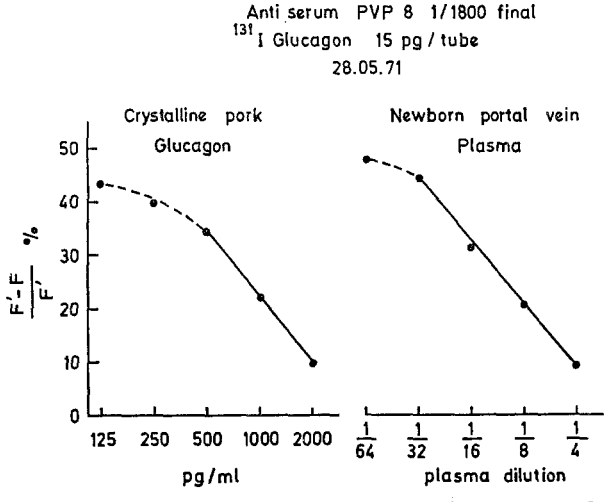

Fig. 2. Dilution slopes of crystalline pork glucagon (on the left) and of a newborn portal vein plasma (on the right). F′ and F see legend to Fig. 1

3. Table 4 illustrates the variations in portal plasma insulin after intraportal glucose. As reported in details elsewhere (Falorni et al., in press), basal portal plasma insulin was significantly higher in IDM; initial insulin response to glucose was markedly enhanced in IDM, statistically higher figures being recorded at +1, +3, +5 and +10 min.

This pattern was not observed in 4 of the 5 IDM who, in contrast, showed a decrease in portal glucagon. Comparison of pooled values measured at the 60th and 90th min of the test revealed a statistically significant difference between controls and IDM: 5020 ± 1647 *versus* 673 ± 99 pg/ml (mean ± S. E. M.) respectively ($p<0.05$).

Discussion

The portal plasma of human neonates contains a material that reacts with glucagon antibodies. Dilution curves of this material are identical to those of crystallized pancreatic glucagon, arguing strongly in favour of its pancreatic origin. The dilution curves of human gut extracts are (indeed) known to differ from those of pancreatic extracts or crystallized glucagon (Samols *et al.*, 1966) when using the so-called "partially discriminant" antisera (Heding, 1972). The absence of detectable amounts of gut GLI in newborn portal vein plasma might be related to the fact that oral glucose is presumably the major factor mobilizing gut "glucagon-like-immunoreactivity" (GLI) both *in vivo* (Samols *et al.*, 1965) and *in vitro* (Luyckx and Lefebvre, 1969) and that, aside from the minimal amount[1] of glucose

1 At the end of a normal pregnancy, the usual concentration of glucose in the amniotic fluid averages 30–35 mg%. If the foetus is conceded to ingest ± 20 ml

ingested with the amniotic fluid, the gastro-intestinal tract of the infants studied had never been in contact with substantial amounts of glucose. Changes in the concentrations of portal plasma glucagon in normal neonates are relatively small, the usual trend being a slight increase between the 6th and the 12th or the 12th and the 18th hours. Although limited, these changes in plasma glucagon might be of importance in regulating liver glycogenolysis and gluconeogenesis during total starvation. It should be recalled in this connexion that both human (Cornblath *et al.*, 1958; de Meyer, 1968) and rat (Girard and Bal, 1970) newborn liver do respond to glucagon by increasing their glucose output. It is too early to speculate about our preliminary finding of very high plasma glucagon levels at certain moments in the portal blood of IDM. More striking are the differences between normal neonates and IDM in their endogenous glucagon response to a massive intravenous glucose load. Normal infants showed no inhibition of glucagon secretion in response to the glucose load. Moreover, cataglycemia[2] was accompanied by a rise in plasma glucagon. In contrast, compared to normal infants, plasma glucagon was significantly lower after exogenous glucose in IDM. In view of the recent data (Unger *et al.*, 1970) suggesting that the entry of glucose into the α cell is an insulin-dependent process and the accepted fact, also confirmed here, that IDM have higher plasma

Table 3. *Portal blood glucose (mg%) after intraportal glucose (1 g/kg BW) in normal infants (upper part) and IDM (lower part)*

Time (min)		0	10	20	30	40	60	90	K-10^{-2}
Normals	B. F.	81	285	268	247	176	125	85	1.00
	C. G.	86	313	266	246		188	110	1.07
	P. S.	66	465	320		209	171	95	1.50
	M. B.	99	332	285	251		180		1.17
	R. M.	52	342		209		132	95	0.91
	T. A.	44	270	230	165		105		1.82
	Ro. Ma.	109	275	218			154	123	0.85
	m	77	326	265	224	193	151	102	1.19
	SEM	9	25	15	16	17	12	7	0.13
I.D.M.	G. A.	66	275	228	190	171	123	88	1.54
	R. S.	60	295	254	195	175	150	86	1.73
	L. G.	61	295	274	228	172	104	57	1.31
	M. V.	32	247	199	152	114	57	38	2.88
	V. C.	33	271	223	228		104	62	1.82
	m	50	277	236	199	158	108	66	1.86
	SEM	7	9	13	14	15	15	9	0.27
							$p < 0.05$		$p < 0.05$

Table 4. *Portal plasma insulin (μU/ml) after intraportal glucose (1 g/Kg BW) in normal infants (upper part) and IDM (lower part)*

Time (min)		0	1	3	5	10	20	30	40	60	90
Normals	B. F.	30	160	95	50	33	44	60	85	95	45
	C. G.	32		123	78	55	95	100		230	110
	P. S.	31	220	140	85	70	200	280	360	280	100
	M. B.	42	136	95	70	72	75	57		105	
	R. M.	30	240	90	52	90		140		170	62
	T. A.	22	110	98	65	56	56	78			95
	Ro. Ma.	40	265	110	60	90	115	130		151	140
	m	32	189	107	66	67	98	121		172	92
	SEM	3	25	7	5	8	23	29		29	14
I.D.M.	G. A.	88	270	190	168	160	165	130	133	140	65
	R. S.	50	500	200	120	220	58	60	62	120	70
	L. G.	100	740	600	180	130	134	120	200	190	80
	M. V.	60	560	390	260	440	280	320	320	130	80
	V. C.	20	560	480	155	137	142	112		255	90
	m	64	526	372	177	217	156	148	179	167	77
	SEM	14	76	80	23	58	36	45	55	25	4
		$p < 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.001$	$p = 0.02$					

of amniotic fluid per hour, the amount of glucose thus ingested could not exceed 6–7 mg/h, i.e. 140–170 mg/day (Assali *et al.*, 1968).

2 The term "cataglycemia" is used to characterize a decline in blood glucose during a given time, without necessarily reaching hypoglycemic values.

insulin levels than normal neonates (Gentz *et al.*, 1967; Pedersen, 1968; Falorni *et al.*, in press) (as evidenced here also by the K values of the two groups), it is tempting to speculate that the absence of cataglycemia-induced glucagon secretion in the IDM might result from the relative hyperinsulinism facilitating glucose entry into the α cells. This hypothesis however remains to be demonstrated.

Table 5. *Portal plasma glucagon (pg/ml) after intraportal glucose (1 g/kg BW) in normal infants (upper part) and IDM (lower part)*

Time (min)		0	10	20	30	60	90
Normals	B. F.	733	695	749	802	894	—
	C. G.	1864	2444	1123	1138	2169	8984
	P. S.	6294	18211	5622	7578	14480	—
	M. B.	1253	1146	1681	1940	1016	—
	R. M.	412	336		512	5072	4889
	T. A.	2162	1883		1756	2657	
	m	2120	4110	2293	2287	4381	6937
	SEM	877	2837	1126	1081	2111	2048
I.D.M.	G. A.	619	550	428	571	405	
	R. S.	527	512		266	428	489
	L. G.	2016	3346	1726	3285	1039	
	M. V.	2169	825		550	993	
	V. C.	435	237		679	618	741
	m	1153	1094		1070	696	615
	SEM	385	570		558	136	126

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