NET INSULIN SECRETION AND IRI RESPONSE PER UNIT OF GLUCOSE DURING HYPOTENSION AND SHOCK

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SUMMARY: In anesthetized dogs after necessary preparations pancreatic blood flow, insulin, concentrations in pancreatic venous and arterial plasma were determined. Net insulin secretion (the difference between inflowing and outflowing insulin per 100 gram of pancreas per minute), the amount of inflowing glucose into the pancreas (mg/100g P/min) and the insulin response to 1 mg of glucose (insulin secretion index) were calculated under control conditions and during hypotention and shock.

Arterial plasma insulin concentration showed a marked increase in hypotension ($p<0.01$) and shock ($p<0.001$) as did pancreatic venous plasma insulin concentration ($p<0.01$). Inflow of glucose to pancreas however was very significantly decreased ($p<0.001$) reflecting the reduced blood flow. Net insulin secretion of pancreas was also reduced significantly during shock ($p<0.05$), while insulin secreted in response to 1 mg of glucose insulin secretion index revealed a statistically to 1 mg of glucose insulin secretion index revealed a statistically important increase ($p<0.01$). Linear correlation was observed between some of these parameters while others revealed non-linear (logarithmic, exponential or hyperbolic) relationships.

Key Words: Insulin secretion, pancreatic blood flow, net insulin secretion, insulin secretion index.

INTRODUCTION

Many investigations of carbohydrate metabolism have been carried out since Claude Bernard (26) observed that hyperglycemia developed during shock (3,11,13,64). To these, peripheral blood insulin levels (5, 43, 50) and insulin secretion (4, 11) during shock have recently been added. Some investigators maintained that insulin secretion or its concentration in the peripheral vessels during shock is increased while others claimed it reduced (11, 13, 21, 45, 48,50). A clear understanding of this problem on the other hand is important from theoretical as well as from practical points of view: If insulin secretion is reduced during shock its supplementation may contribute significantly to the intracellular metabolism and perhaps to the development of irreversibility of shock (73). The very treatment will be superfluous on the other hand if insulin secretion is not reduced during shock. Furthermore peripheral insulin levels may not be indicative of insulin secretion (7,9). These problems are investigated in a series of animals in hypotension and shock and are presented in this communication.

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MATERIALS AND METHODS

31 mongrel dogs of either sex weighing 15-30 kg were used for these experiments. After an overnight fast the animals were anesthetized with sodium pentobarbital, 25 mg/kg iv an after tracheal intubation the right femoral artery was catheterized for continuous monitoring of intraarterial pressure and for blood sampling. The abdomen was opened by a midline incision. The pancreas was exposed and polyethylene catheters with an inner diameter of approximately 1 mm were placed into small

branches of the superior pancreatic artery and into an adjoining vein. The small catheter in the artery was for ^{133}Xe injection, and the one in the vein to obtain blood samples. Extra care was taken to avoid hemorrhage and petechia into the pancreas. A glass cannula was inserted into the left femoral artery and was connected to a Lamson bottle and a mercury manometer with an appropriate polyethylene catheter. The inner compartment of the Lamson bottle was connected to a reservoir with a volume of 30 liters forming an airtight system. The pressure in this closed system was adjusted to the desired level by means of a pump connected to the reservoir. Water at 38°C was continuously circulated through the outer compartment of the Lamson bottle to maintain the temperature at 38°C into which twenty mg of heparin was added to avoid coagulation. The blood was constantly stirred to prevent sedimentation. Systemic arterial blood pressure was monitored with anotler mercury manometer throughout the experiment.

After the catheters were placed into the vessel they were filled with heparinized saline (10 U/ml). Before blood samples were taken the fluid in the catheters and some blood were withdrawn and discarded to avoid dilution of aliquots.

From the time of completion of the operative procedures a period of 20 min was allowed for adaptation of the animal and diminution of the stress effect. 0.1-0.2 ml saline containing 100-300 uCi of ^{133}Xe was injected into the catheterized pancreas artery followed by 0.2-0.3 ml heparinized saline to replace the radioactive remnants in the catheter. The clearance of the tracer from the pancreas was monitored by Nuclear Chicago Model 2524, Renaltron IV System. Blood samples were then taken for initial studies (stage I) and the animals were separated into two groups at random. In the experimental group there were 19 dogs, which after the above preparations were bled into the Lamson bottle and the peripheral arterial blood pressure was reduced stepwise to 80, 60 and 30 mm Hg, corresponding to the stages II, III, and IV of the experiment respectively. At cach level of the blood pressure 30 min was allowed to obtain a state of equilibrium before sampling and subsequent procedures. 12 dogs in the control group were treated likewise except for exsanguinution.

Blood aliquots were taken from the femoral artery and from the pancreatic vein at each stage of the experiment. Blood pressure, pulse, respiration and the depth of anesthesia were carefully monitored throughout the study. Blood glucose was measured by Somogy-Nelson, and hematocrit was determined using micro method (Picker, Y.S.I. model 30). Plasma insulin levels were determined by radioimmunoassay method of Hales and Randle (32). The pancreatic blood flow rates were calculated according to the methods described earlier (11, 24).

The amount of inflowing glucose into the pancreas, net insulin secretion and the insulin response to 1 mg of glucose were calculated at each stage of the experiment using the following formulas:

$$\text{Pancreatic Plasma Flow} = \frac{(100 - \text{Hct}) \times \text{PBF}}{100} \text{ (ml /100 g P/min)}$$

Amount of Net Insulin Secretion = PPF x (Pvi - Pai) (uU)100 g P/min)

$$\frac{\text{Amount of Blood Glucose Perfusing}}{\text{100 g Pancreas Tissue}} = \frac{\text{Pag} \times \text{PBF}}{100} \text{ (ml /100 g P/min)}$$

$$\frac{\text{IRI response to 1mg of glucose or (Insulin secretion index)}}{} = \frac{\text{Amount of Net Insulin Secretion}}{\text{Amount of Glucose Coming into Pancreas}} \text{ (ì U/mg)}$$

PBF = Pancreatic Blood Flow
PPF = Pancreatic Plasma Flow
Hct = Hematocrit
Pvi = Pancreatic Vein Insulin Concentration
Pai = Arterial Plasma Insulin Concentration
Pag = Pancreatic Artery Blood Glucose Concentration

The means and standard deviations were calculated and the statistical significance between groups were studied according to the student's t test. The findings of each step in both group were also compared with each other as well as with the initial values. All the statistical evaluations were done by the Electronic Data Processing Center of Hacettepe University using Burroughs 6800 Computer.

RESULTS

Changes in Blood Pressure

The beginning mean arterial blood pressure of the control group was 136.4 ± 3.4 mm Hg and it was maintained within ±10mm Hg throughout the observation period. In the experimental group the mean blood pressure at the beginning was 132.8 ± 2.6 mm Hg and was not significantly different from the control group ($p>0.05$). The blood pressure of the dogs, in the experimental group was gradually reduced and then maintained at predetermined levels as outlined above. If however the blood pressure of any animal tended to go into spontaneous hypontension or shock it was eliminated from the series.

Arterial Blood Glucose Concentration

Arterial blood glucose concentration of all cases combined was 86.5 ± 1.6 mg % during stage I (Table 1). In control series its concentration gradually rose and attained statistical importance at stage III ($p<0.01$) and at stage IV ($p<0.001$). In the experimental group the rise was more impressive and very significantly different from that of the

Table 1

	Control Group				Experimental Group		
	Stage I	Stage II	Stage III	Stage IV	Stage II	Stage III	Stage IV
Femoral arterial blood glucose (mg/100ml)	85.6 ± 1.6	91.5 ± 2.9a_1	98.0 ± 2.8 xx,b_1	102.4 ± 3.7 xxx,a_3	114.7 ± 4.6 xxx,a_1	144.3 ± 8.2 xxx, b_1	217.3 ± 18.5 xxx, a_3
Peripheral venous plasma insulin concentration (μU/ml)	8.6 ± 0.9	6.4 ± 1.2a	6.5 ± 1.3 a_1	8.5 ± 1.7a_2	16.9 ± 3.0 x, a	21.3 ± 3.3 xxx, a_1	37.9 ± 7.7 xxx, a_2
Pancreatic vein plasma insulin concentration (μU/ml)	204.1 ± 23.9	270.5 ± 54.8	277.5 ± 48.6	279.0 ± 50.8	283.5 ± 52.1	418.3 ± 72.7 xx	425.2 ± 91.3 xx
Pancreatic blood flow rate (ml/100 g P/min)	83.5 ± 7.5	73.0 ± 9.4a_2	74.6 ± 8.7 a_3	79.4 ± 10.4 b_3	49.8 ± 3.7 xxx, a_2	29.3 ± 2.3 xxx, a_3	20.2 ± 2.3 xxx, b_3

x ($p<0.02$), xx ($p<0.01$), xxx ($p<0.001$) represent statistically significant variation of the figure so marked compared to stage I (initial value) of the same parameter. a ($p<0.05$), a_1 and b_1 ($p<0.01$), a_2 ($p<0.02$), a_3 and b_3 ($p<0.001$) indicate statistically significant variations between identically marked stages of the same parameter of both groups.

control group during all stages of the observation ($p<0.001$).

Plasma Insulin Concentrations

a. Arterial plasma insulin concentration: After preparation of the experiment the mean arterial plasma insulin concentration of both groups combined was 8.6 ± 0.9 μU/ml (Table 1). In the control group this parameter revealed minor variations throughout the experiment ($p>0.05$). In the experimental group the arterial plasma insulin concentration rose significantly soon after exsanguination and went on rising until the end of the observation ($p<0.001$).

b. Pancreatic vein plasma insulin concentration: Insulin concentration in the pancreatic vein was 304.1 ± 23.9 μU/ml at stage I (Table 1). It gradually rose during the observation period but never attained statistical significance in the control group. In the experimental group the rise was more accentuated and was statistically important at stage III and IV ($p<0.01$).

Pancreatic Blood Flow Rate

The pancreatic blood flow rate of both groups combined was 83.5 ± 7.5 ml/100 g P/min at the initial stage (Table 1). There was in this parameter a slight reduction in the control group at the later stages of the observation. It did not, however, reach statistically significant level. In the experimental group the reduction was more impressive during all stages of the observation ($p<0.001$).

Inflowing Glucose

The mean inflow of glucose to 100 g of pancreas per minute was 73.4 ± 5.5 mg/100 g pancreas/min at stage I (Table 2). There was no significant variation in the amount of glucose inflowing to pancreas in the control group. In the experimental group the inflow glucose was obviously reduced at stage II. This trend became much more impressive later, attaining statistical significance at stage III and IV ($p<0.001$).

Net Insulin Secretion

Net insulin secretion (the difference between inflowing and outflowing insulin per 100 g pancreas per minute) was 9.6 ± 1.6 mU/100g P/min) during stage I (Table 2). A slight but steady increase was observed in this parameter throughout the study in the control series ($p>0.05$). In the experimental group the net insulin secretion dropped gradually but consistently, reaching statistical significance at stage IV ($p<0.02$).

IRI response per unit of glucose or insulin secretion index

Under control conditions the mean value of IRI response to 1 mg of glucose perfusing the pancreas was found to be 124.5 ± 17.9 μU/mg at stage I. Of this parameter none of the changes observed at any stage of either group was found statistically different when compared with the corresponding initial value or between the groups (Table 2). The results of each stage was then calculated

Table 2

	Control Group				Experimental Group		
	Stage I	Stage II	StageIII	Stage IV	Stage II	Stage III	Stage IV
Inflow of glucose to pancreas (mg/100 g/min)	73.4 ± 5.5	71.5 ± 11.7	77.6 ± 11.8 a_1	81.9 ± 14.1a_2	57.2 ± 5.5	39.9 ± 4.9 xxx, a_1	40.5 ± 6.9 xxx,a_2
Net insulin secretion of the pancreas (mU/100 g/min)	9.6 ± 1.6	12.8 ± 4.4	12.6 ± 3.7	14.1 ± 5.1 a_3	7.7 ± 1.9	7.0 ± 1.8	4.3 ± 0.9 x, a_3
Insulin response to 1mg glucose (μU/mg)	124.5 ± 17.9 (100.0 ± 0.0)	166.9 ± 31.8 (110.3 ± 12.1)	166.6 ± 33.3 (106.3 ± 10.0)	168.4 ± 33.9 (109.1 ± 9.9)	128.3 ± 27.3 (168.8 ± 39.5)	172.6 ± 34.7 (258.1 ± 61.3)	110.4 ± 16.1 (192.4 ± 67.3)

x ($p<0.02$) and xxx ($p<0.01$) indicate statistical significance compared to stage I of the same parameter.

a_1 ($p<0.05$) and a_2 ($p<0.01$), and a_3 ($p<0.001$) indicate statistical significance between the identically marked stages of the control and experimental groups.

as the percentile of its own value for each individual animal and its variations throughout the observation were figured out. Their means and standard errors were than calculated (Table 2, figures in parenthesis). It was then observed that plasma insulin concentration was 110.3 ± 12.1, 106.3 ± 10.0 and 109.1 ± 9.9% of the initial value at stages II, III and IV of the control group. They were still statistically insignificant. In the experimental group, however, the figures of the corresponding stages rose to 168.8 ± 39.5, 258.1 ± 61.3 and to 192.4 ± 67.3% of the initial value the last two of which attained statistical significance ($p<001$ and $p< 0.05$ respectively).

Correlation studies between the parameters

In order to understand the relationship between these parameters detailed correlation studies were carried out (Table 3). Although the correlation coefficients were mostly rather high, there actually was linear relationship between only some of them. Regression studies were performed on the remaining figures at which time nonlinear relations were uncovered between many parameters (logarithmic, hyperbolic or exponential).

DISCUSSION

The experimental preparation used in this investigation is appropriate for insulin secretion studies (10). It has the avantage of keeping the pancreas in-situ, and therefore, preserving intact the nervous (6,9,57), the endocrin (2, 43) and the metabolic (25,28,39,63,72) factors important for regulation of insulin secretion. The knowledge so obtained is, therefore, more relevant to the clinical conditions (10,11). It is furthermore complementary of the data obtained from in vitro or in situ perfused pancreas and isolated Langerhans islands used frequently in recent years (38,44,53,67).

Gradual rise observed in arterial glucose concentration in the control series (Table 1) represents the effect of stress and anesthesia and has formerly been reported by several authors (5,11,13,25,33,34). The insulin concentration in the pancreatic vein rises concurrently indicating that the B cell has responded to the rising blood sugar in the expected manner. Since the peripheral venous insulin level is even somewhat reduced this reaction has not reflected to the peripheral circulation. The reason for this must of necessity be the increased uptake of insulin by the liver stimulated by the high adrenalin levels encountered under conditions of stress (4,64).

There is voluminous literature confirming that the most important stimulant of insulin synthesis and secretion is glucose (1,9,49,58,62,70,77). This is further confirmed by the result of our shock series where a very significant correlation is observed between the femoral arterial glucose and the peripheral venous insulin concentrations (Table 3). In the control series on the other hand this relationship is reversed. Inflow of glucose to pancreas was then considered and observed that this parameter revealed a small rise in the control series (Table 2). A correlation therefore existed between them, which was also reversed in cases of shock.

It is interesting that there was a moderate correlation between the glucose inflow to the pancreas and the pancreatic vein insulin concentration in the control cases, while a strong but negative correlation occurred in cases of shock. It is also noteworthy that a negative relationship existed between the femoral venous plasma insulin concentration and inflow of glucose for both groups. The data

Table 3

	$FVPIC_C$	$FVPIC_S$	$PVPIC_C$	$PVPIC_S$	IGP_C	IGP_S	NIS_C	NIS_S	$IRImgG_C$	$IRImgG_S$	BPF_C	BPF_S
$FAGC_C$	-0.77		0.85		0.87		0.90		0.81		0.33	
$FAGC_S$		0.99		0.86		-0.83		-0.99		-0.22		-0.88
$FVPIC_C$			-0.99		-0.35		-0.92		-0.99		0.84	
$FVPIC_S$				0.85		-0.82		-0.99		-0.25		-0.89
$PVPIC_C$					0.48		0.95		0.99		-0.77	
$PVPIC_S$						-0.99		-0.87		0.30		-0.97
IGP_C							0.59		0.41		0.17	
IGP_S								0.86		-0.33		0.99
NIS_C									0.95		-0.58	
NIS_S										0.20		0.92
$IRImgG_C$											-0.80	
$IRImgG_S$												-0.18

FAGC : Femoral arterial glucose concentration (mg/100ml)

FVPIC : Femoral venous plasma insulin concentration (μU/ml)

PIVPIC : Pancreatic vein plasma insulin concentration (μU/ml)

IGP : Inflow of glucose to pancreas (mg/100g P/min)

NIS : Net insulin secretion (μU/100g P/min)

IRImgG : Insulin response to 1 mg of glucose arriving to pancreas/min

PBF : Pancreatic blood flow (ml/100g P/min)

c and s subletters represent values of control or experimental series respectively.

in Table 2 indicate that the net insulin secretion rose slowly but steadily in animals under anesthesia, a response closely correlated with the femoral arterial glucose concentration, with the inflow of glucose to pancreas and with the pancreatic vein insulin concentration under control conditions. This relationship is reversed in shock for the former two parameters. It remained however positively correlated between the inflow of glucose to pancreas and net insulin secretion during shock (Table 3).

Reduced blood flow to pancreas in hemorrhagic shock has formerly been reported by many investigators (11, 60,75) and is not therefore an unexpected observation. The pancreatic blood flow rate is positively correlated with the femoral vein plasma insulin concentration in control cases. It is also positively correlated with the net insulin secretion and with the inflow of glucose in cases of shock but negatively correlated with the pancreatic vein insulin concentration in both series, the femoral arterial blood glucose concentration and the insulin response to 1 mg of glucose as well as with insulin concentration in femoral vein in experimental series. Even though one would expect a positive correlation between these parameters, the declining blood flow to pancreas observed in both series seems to have produced inverse relationships.

At this point we considered whether the changes in insulin response to 1 mg of glucose might explain the alterations of glucose inflow to pancreas which would contribute to regulation of insulin secretion. We therefore calculated the response of the pancreas to 1 mg of glucose which we propose to call the "insulin secretion index". It was noted that the B cell responded to the gradually climbing blood sugar in the control series by secreting more insulin during the entire observation period compared to the initial stage. Since the femoral arterial glucose concentration was simultaneously rising (Table 1) these two parameters remained positively correlated in the control but not in the experimental series. During stage III of the experiment the insulin response to 1 mg of glucose was significantly increased. Later at the irreversible stage it revealed a downward course. This trend appears logical to us, because it is natural that during the latter stages of shock despite more intense stimulation, not as much

insulin synthesis may occur compared to the early stages of the disease and, therefore, less insulin secretion may be possible (4,13).

Insulin response to 1 mg of glucose (9) or insulin secretion index should be considered in comparison with the insulinogenic index (51,63). The latter is calculated using the insulin concentration in the peripheral blood. It cannot, therefore, be sufficiently indicative of insulin secretion (7,9,10,42). It is furthermore known that several factors other than the amount of insulin secreted per minute of time alters the insulin concentration of the peripheral venous blood. Among these, the changes of the blood volume in which it is diluted, variations of glucose and insulin uptake by the liver and the peripheral tissues may be mentioned (9,10). We therefore, believe that the net insulin secretion and insulin response to 1 mg of glucose are more efficient means of evaluating the "insulin secretion".

We may conclude from the data reviewed up to this point that glucose alone can not explain the changes in insulin secretion. The variable nature of correlation between many parameters studied deserves further consideration. Even though pancreatic blood flow rate with its course contrary to some parameters offers a change of explaining some of these unexpected observations, it also entails many functional connotations: Reduced blood flow to pancreas means that lesser amount of nutritients, metabolites, hormones, oxygen and also glucose become available to the B cell. This would naturally result in a reduction in the amount of insulin synthesis and inadequately functioning mechanims of secretion (46,47).

Reviewing the data recorded in the literature concerning the relationship between glucose and insulin one observes an interesting development: Turner *et al.* (70) uncovered a linear relation between glucose concentration and insulin secretion. Fischer (25), however, observed that the peripheral blood glucose areas are related to insulin (IRI) concentration areas but independent of stimulated IRI output rate. Porte *et al.* reported earlier that infusions of epinephrine in normal man incited severe hyperglycemia while suppressing insulin response (57). Efendic *et al.* observed that all stages of glucose intolerance are accompanied by a decreased ability of glucose to initiate insulin release by a diminished sensitivity to insulin (23). These conclusions suggest that the relationship between the blood glucose and insulin is not linear under all conditions, it may rather be subject to change. This was also observed by Metz who found the result to fit to a logarithmic curve (49). Menguid on the other hand observed that this relation between glucose and insulin was not even apparent under some conditions (48).

Other investigators claimed that an optimal glucose concentration is needed for stimulation of insulin synthesis and secretion by many substrates as well as other secretagouges (53, 76) which means that one factor modifies the response of the B cell to another. In fact these influences appear to be multifactorial as suggested by the following studies: Haring *et al.* (36) suggested that the insulin receptor is regulated not only by serum insulin level but also by cortisol. Sanson *et al.* (61) showed that glucose dependent insulinotropic polypeptide potentiates the release of insulin only when glucose level is elevated. Whittaker and Taylor (72) found no effect of growth hormone on the release of insulin from freshly isolated islets during 30 minute incubation periods. By contrast islets previously cultured for 16 hours with growth hormone exhibited a 40% increase in the release of insulin in response to glucose or to glucose and theophylline. In addition and long before any of these investigators Claude Bernard produced diabetes mellitus in dogs by puncturing the base of fourth ventricle (26). This is the first observation indicating that the central nervous system played a crucial role in regulation of carbohydrate metabolism. In later years this function of the central nervous system was supported by clinical and experimental data: It is well known by the clinicians that physical or emotional trauma often leads to prelude of some endocrin disorders or decompensation of the previously existing diseases. Among these the best known is the development of acidosis which may even lead to diabetic coma. Lesions of ventromedial nuclei (28,29,58,65), as well as the influences of enkephalins (30) also indicate that nervous system most likely play important roles in regulation of insulin secretion (68). Experiments where glucose was injected into the internal carotid artery (16) or insulin was injected into the left lateral ventricle (15) also support this belief. Furthermore adrenergic stimulation among other factors influencing the response of the B cell have long been known to alter insulin secretion (5,6,8, 57,67, 68). That central nervous system also has a significant part in producing the peripheral hyperglycemia has been well documented by its correlation with hepatic glycogenolysis (22).

Glucose compared to the above referred indirect effect acts directly on the B cell and thereby contributes to regulation of insulin secretion (2,53,69). There are other factors also acting directly on the B cell among which the ovarian hormones (3,38,43,66), dopamine and serotonine (77), insulinotrcpic polypeptide (61), extracts of intestinal mucosa (18), cholecystokinin and other hormones (20,38,72,74) and many drugs (14,30,62) modify insulin secretion. They also act directly on the B cell causing

alterations of the insulin synthesis and its releasing mechanisms, a view supported by many studies conducted on isolated in situ pancreas (17), *in vitro* incubated pancreas or its portions (18,53) and *in vitro* incubated pancreas islets (72). Other nutrients (39,42,74) also operate directly on the B cell.

Besides these extraneous causes for a changing behavior of the B cell during shock intrinsic factors also exist. Among them we may mention the observations concerning the variations of ultrastructural organells (54), which may of necessity involve alterations in the enzyme activities (2,31,55). It should not be forgotten that changes in the receptors of the B cell during shock may entail great functional importance (19,27,41,52,59). Both of these cellular changes would naturally lead to significant metabolic derangements which in turn would result in abnormalities of mechanisms responsible of insulin synthesis and secretion and thereby contribute to development of the non-linear correlations observed in our series of experiments (33).

We may also consider the next and most frequently used other method frequently been used for determination of insulin secretion is estimation of protein C levels in the circulating blood (35,40,56). We have planned but have not as yet been able to compare these two methods. It should be considered however that ours is a direct method possible sources of error of which are limited to those of radioimmunoassay of insulin and of measurement of blood flow (9,10). Metabolic details of C peptide on the other hand may introduce a more complicated pattern and the results may therefore reflect significant inaccuracies. To this effect it is important to refer to the recent work where protein C levels have not been found in correlation with hemoglobin A_{1c} levels (71).

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