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Growth Hormone and Metasomatotrophic Diabetes: Effects on Insulin and Proinsulin of Serum and Pancreas in Dogs

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Summary. In normal fasting dog serum, the insulin: proinsulin molar proportion was 71:29%. In response to glucose infusion, the proinsulin proportion decreased. In the pancreas, the proinsulin proportion was lower than in serum. Growth hormone treatment for one day increased serum insulin sevenfold and proinsulin 18-fold. The proinsulin proportion increased to 49%. The growth hormone injections magnified the response to glucose infusion. The rise in serum insulin was 16 times the normal, proinsulin also rose but its proportion decreased. Growth hormone treatment for 6 days decreased pancreatic insulin to 5% and proinsulin to 46% of normal. In the permanent (metasomatotrophic) diabetes produced by the prolonged administration of growth hormone, serum insulin decreased and the proinsulin proportion increased. No rises in serum insulin nor proinsulin occurred following glucose infusion. In the pancreas, insulin and proinsulin were reduced to 1.6% and 8% of normal. The reduction in the immunoreactive insulin of the pancreas was more pronounced in the tail than in the head and body regions. The results indicate that in the state of augmented insulin secretion and hyperinsulinaemia produced by growth hormone and in the reduced insulin secretion and hypoinsulinaemia of metasomatotrophic diabetes, the proportion of proinsulin in serum is increased due to beta cell secretion containing a higher proportion of proinsulin than normal.

Key words: Growth hormone effects, somatotrophic diabetes, metasomatotrophic diabetes, hyperinsulinaemia, hyperproinsulinaemia, hypoinsulinaemia, pancreatic insulin and proinsulin

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In the blood of healthy subjects, proinsulin forms a substantial part of the immunoreactive insulin (IRI) [1]. In the pancreas, however, the proportion of proinsulin is lower [1, 2]. The roles of the secretion and clearance rates of insulin and proinsulin in the production of this difference between serum and pancreas have been investigated [3]. The proportion of proinsulin in blood is high in some states or cases in which insulin secretion is increased, as in obesity [4], in patients with insulinoma [5–8] and in tolbutamide-treated rats [9]. In certain types of diabetes mellitus, the proinsulin proportion is also elevated [1, 10–14].

Growth hormone has the capability of increasing insulin secretion in man [15, 16] and animals [17–19]. However, in the permanent (metasomatotrophic) diabetes produced by the prolonged administration of growth hormone, insulin secretion is low [20]. In extension of our investigations [20, 21] we now describe the effects of growth hormone and of metasomatotrophic diabetes in dogs on the insulin and proinsulin of serum and pancreas, under the conditions of fasting and glucose infusion.

Materials and Methods

Normal adult male dogs were given daily 425 g canned meat (Romar Pet Foods, Toronto) and 200 g dog chow (Purina, Toronto) divided in two meals. The response to glucose was tested about 17 h after a meal by IV injection of glucose (1.0 g/kg body weight as a 50% solution of glucose in 0.154 mol/l NaCl). In a group of six dogs (initial body weight 15–20 kg, mean serum IRI 23 mU/l and glucose 4.6 mmol/l), bovine growth hormone (Connaught Medical Research Laboratories, Toronto, Lot 100-1, dissolved in 0.154 mol/l NaCl, 10 mg/ml, at pH 8–9) was injected SC twice daily. The dose per day was 2.0 mg/kg body weight for 2 days and 1.0 mg/kg for the remaining 4 days. At 1 day after the first of these injections, serum IRI increased 20-fold: after 2 days serum glucose also rose and all the dogs excreted sugar. The hyperinsulinaemia, hyperglycaemia and glycosuria continued to the time of sacrifice, at 6 days, when the mean serum IRI was 418 mU/l, glucose was 20.2 mmol/l and sugar excretion was about 25 g/day [21].

In another group of five dogs (initial body weight 13–18 kg, mean serum IRI 24 mU/l and glucose 4.7 mmol/l) growth hormone injected for 33–44 days (2.0 mg/kg for 10–13 days and 2.6–3.3 mg/kg thereafter) produced permanent diabetes. After cessation of these injections, serum IRI became subnormal after 2–4 months, serum glucose remained high (17.7–21.1 mmol/l) and sugar excretion was about 100 g/day during about 14 months of observation [20].

After 6 days of growth hormone injection and about 14 months of metasomatotrophic diabetes, the dogs were anaesthetized (sodium pentobarbital, 25–30 mg/kg, IV) blood was rapidly withdrawn through a carotid cannula and the excised pancreas was divided into the head (uncinate), body (duodenal) and tail (splenic) regions. The tissue was frozen and later extracted and partially purified according to [22], as described previously [23]. The alcohol-ethyl ether percipitate was dissolved in 0.5 ml of 0.01 mol/l HCl in 0.154 mol/l NaCl. Freeze-dried aliquots of serum (5.0 ml from metasomatotrophic diabetic and fasting normal dogs and 2.0 ml from growth hormone treated and normal dogs post-glucose) were dissolved in 1.0 ml of distilled water, then extracted as above.

Methods for the partition of extracts by gel filtration and the identification and assay of the insulin and proinsulin-like material (PLM) fractions have been described [3–6, 10, 12]. The fractionation column (1.0 × 60 cm) contained Sephadex G 50 superfine (Pharmacia, Uppsala) suspended in 1.0 mol/l acetic acid. which was also the fluid for elution and washing. To minimize absorption, 1.0 ml of normal dog serum was passed through the column. Bovine insulin (Connaught Medical Research Laboratories, Fraction C, free of proinsulin) and porcine proinsulin (Lilly Laboratories, Indianapolis, Lot No. 615-1082B-2) were labelled with 125-iodine (Amersham-Searle, Arlington Heights, Illinois, Reagent IMS-30) [24]. The column was calibrated by the application of these labelled and the unlabelled hormones, in several combinations. Two major peaks of radioactivity coincided with the peaks of immunoreactivity, at samples 35 (proinsulin) and 45 (insulin), with satisfactory separation and recovery of about 85% of the amount applied. In analysis, trace amounts of ^{125}I-insulin (16 pg) and of ^{125}I-proinsulin (44 pg) that were far below the level of detection by radioimmunoassay, were added to the extracts of serum and pancreas, prior to gel filtration. The peaks of radioactivity and of immunoreactivity corresponded. Eluate samples (0.57 ml) under the peaks were pooled, freeze-dried and, for assay, were dissolved in phosphate buffer (0.05 mol/l, pH 7.4) containing bovine serum albumin, 5.0 g/l.

Chromatographic fractions of dog pancreas extract, containing labelled insulin and proinsulin, were examined by electrophoresis in 7% polyacrylamide gel (pH 8.9) at 3 mA/tube [25]. The gel column of the PLM fraction had a zone of the same mobility as porcine proinsulin and several zones of greater and lesser mobility. The insulin fraction gel had a zone corresponding to porcine insulin, but no proinsulin zone.

IRI was assayed by a double-antibody method [26], using as reference standard porcine insulin (Lilly Laboratories, 26.2 U/mg). The serum IRI values obtained with this standard were found in further studies to be in agreement with those given by assays against canine insulin (Novo Laboratories, Copenhagen). The antibody-binding of porcine proinsulin (Lilly Laboratories, Lot 615-7082B-46-2) was found to be about 40% of that of porcine insulin, in fair agreement with others [13, 27]. Porcine C-peptide (Lilly Laboratories, Lot 615-1070B-72) in concentrations of 0.25–20.0 ng/ml, did not react in the assay system, indicating that insulin was the reactive portion of the molecule. The chromatographic PLM fraction was assayed against porcine proinsulin. The antibody-binding of the PLM fraction over a wide range of dilutions was proportional to that of the proinsulin standard, in the concentrations of 0.25–20.0 ng/ml. The molecular weight of canine proinsulin (8855) was obtained as the sum of its C-peptide (mol. wt. 3077, from the amino acid composition) [28] and insulin (mol. wt. 5778). With the assumption that canine and porcine proinsulins reacted equally in the assay system, 1 μg of canine proinsulin corresponded to 113 pmol. Serum glucose was determined by means of glucose oxidase and a polarographic electrode [29]. Values are the means ± SEM: the probability of no real difference between means is given for values < 0.05.

Results

Normal Dogs

In a group of six normal dogs, fasting serum insulin was 141 pmol/l (equivalent to 21 mU/l) and proinsulin was 52 pmol/l (equivalent to 0.46 μg/l). The molar proportion of proinsulin was 29%. In these normal dogs, post-glucose (at 15 min after IV injection 1 g glucose/kg body weight) serum insulin rose significantly by +508 pmol/l over the fasting level, while mean serum proinsulin did not increase significantly (increase in three, no change in one and decrease in two dogs). The molar proportion of proinsulin was 9% (Table 1).

Effects of Growth Hormone

After 1 day of growth hormone injection, fasting serum insulin and proinsulin increased significantly to about seven and 18 times the normal, respectively. The proinsulin proportion increased significantly to 49%, due to the greater rise in proinsulin than in insulin (Table 1). In response to IV glucose, serum insulin rose significantly by +8150 pmol/l, over the already high fasting value: this increment was 16 times the normal response. Serum proinsulin also rose after IV glucose by +540 pmol/l. The greater post-glucose rise in insulin than in proinsulin significantly increased the proportion of insulin to 84% (Table 1).

Metasomatotrophic Diabetes

In metasomatotrophic diabetes, 30 days after the cessation of the growth hormone injections, serum insulin was significantly below normal. The serum proinsulin level was in the normal range, but the proportion was significantly increased (Table 2). Following IV glucose, no rises in serum insulin nor proinsulin occured and the proportion of proinsulin remained high (Table 2).

Pancreas

In normal dogs, the tail region of the pancreas had the highest concentration and amount of IRI. The body and head regions followed in order (Table 3). The insulin and proinsulin concentrations in the whole pancreas extract were 16 and 0.24 nmol/g of tissue, giving the proinsulin proportion of 2% (Table 4). Growth hormone treatment for 6 days reduced the IRI concentrations in the head, body and tail regions of the pancreas to about 11, 10 and 8% of normal, respec-

Table 1. Serum insulin and proinsulin in normal and growth hormone-treated dogs, under fasting and post-glucose (at 15 min after IV glucose) conditions

Dogs	No. of dogs	Conditions	Insulin (pmol/l)	Proinsulin (pmol/l)	Proinsulin (mol %)
Normal	6	Fasting	141 ± 27	52 ± 9	29 ± 5
Normal	6	Post-glucose	649 ± 169	54 ± 10	9 ± 2
Growth hormone (1 day)	6	Fasting	924 ± 225	956 ± 313	49 ± 4
Growth hormone (1 day)	6	Post-glucose	9070 ± 1620	1500 ± 336	16 ± 4
Comparisons					
Normal, fasting vs normal, post-glucose			$p<0.025$		$p<0.010$
Normal, fasting vs GH, fasting			$p<0.010$	$p<0.025$	$p<0.025$
Normal, fasting vs GH, post-glucose			$p<0.005$	$p<0.010$	
GH, fasting vs GH, post-glucose			$p<0.005$		$p<0.001$
Normal, post-glucose vs GH, post-glucose			$p<0.001$	$p<0.010$	

Values are expressed as mean ± SEM.
GH = growth hormone injected

Table 2. Serum insulin and proinsulin in metasomatotrophic diabetic dogs under fasting and post-glucose (at 15 min after IV glucose) conditions

Dogs	No. of dogs	Conditions	Insulin (pmol/l)	Proinsulin (pmol/l)	Proinsulin (mol %)
Metasomatotrophic diabetic (30 days)	5	Fasting	71 ± 8	80 ± 11	53 ± 5
Metasomatotrophic diabetic (30 days)	5	Post-glucose	50 ± 4	50 ± 8	49 ± 5
Comparisons					
MSD, fasting vs normal, fasting			$p<0.050$		$p<0.025$
MSD, fasting vs GH, fasting			$p<0.005$	$p<0.025$	
MSD, post-glucose vs normal, fasting			$p<0.010$		$p<0.025$
MSD, post-glucose vs normal, post-glucose			$p<0.010$		$p<0.001$
MSD, post-glucose vs GH, post-glucose			$p<0.001$	$p<0.005$	$p<0.001$

Values are expressed as mean ± SEM
GH = growth hormone injected; MSD = metasomatotrophic diabetic

Table 3. Distribution of immunoreactive insulin (IRI) in the pancreas

Dogs	No. of dogs	Body weight (kg)	Pancreas weight (g/kg body weight)	IRI in regions of the pancreas					
				Head		Body		Tail	
				(U/g of tissue)	(%)[a]	(U/g of tissue)	(%)	(U/g of tissue)	(%)
Normal	3	15.2 ± 2.2	2.2 ± 0.1	2.3 ± 0.74	13 ± 2.5	2.6 ± 0.94	34 ± 3.4	4.8 ± 1.9	52 ± 3.7
Growth hormone (6 days)	6	18.2 ± 0.9	1.6 ± 0.2	0.26 ± 0.07	22 ± 3.3	0.26 ± 0.07	42 ± 3.6	0.37 ± 0.17	36 ± 4.3
Metasomatotrophic diabetic (14 months)	5	13.2 ± 0.9	1.9 ± 0.3	0.044 ± 0.005	23 ± 1.1	0.056 ± 0.013	43 ± 5.3	0.044 ± 0.008	34 ± 4.5
Growth hormone vs metasomatotrophic diabetic				$p<0.025$		$p<0.05$			

Values are expressed as mean ± SEM
[a] IRI of the region as precentage of the total pancreatic IRI

tively (Table 3). The insulin and proinsulin concentrations in the whole pancreas extract were reduced to about 5 and 46% of normal, respectively. The greater reduction in insulin than proinsulin increased the proinsulin proportion to 11% (Table 4).

In metasomatotrophic diabetes of 14 months duration in five dogs, the IRI of the head, body and tail regions of the pancreas was reduced to about 1.9, 2.1 and 0.92% of normal respectively (Table 3). The insulin and proinsulin concentrations in the whole pan-

Table 4. Insulin and proinsulin in pancreatic tissue of normal, somatotrophic diabetic and metasomatotrophic diabetic dogs

Dogs	No. of dogs	Insulin (nmol/g)	Proinsulin (nmol/g)	Proinsulin (mol %)
Normal	3	16 ± 9	0.24 ± 0.14	2 ± 0.3
Growth hormone (6 days)	6	0.82 ± 0.33	0.11 ± 0.03	11 ± 2
Metasomatotrophic diabetic (14 months)	5	0.25 ± 0.08	0.019 ± 0.008	10 ± 1

Values are expressed as mean ± SEM

creas extract were reduced to about 1.6 and 8% of normal and the proportion of proinsulin remained high (Table 4).

Discussion

Insulin and proinsulin were present in normal fasting dog serum in the molar proportion of 71 : 29%, while in the pancreas the proportion of proinsulin was much lower. Comparable relations in man have been attributed to the secretion of IRI similar in composition to that contained in the pancreas and to the higher rate of clearance of insulin than of proinsulin from the systemic and portal circulations [1, 3, 30].

The rise in serum IRI produced by growth hormone in the dog [21, 31, 32] is now shown to be due to elevation in both insulin and proinsulin. The extent and promptitude of these rises (seven- and 18-fold, respectively, after one day of treatment) are indicative of increased rates of secretion. While the influence of growth hormone on the clearance of these peptides from the circulation is not yet known, the absence of change in the systemic clearance of IRI [33] suggests that this factor may not be of primary importance. The increased proportion of proinsulin in serum produced by growth hormone suggests that the rate of secretion of proinsulin by the pancreatic beta cells was increased to a greater extent than that of insulin.

Growth hormone administered for several days to dogs depletes the beta cells of granules [34] and the pancreas of its insulin content [21, 31]. The accompanying increase in the proinsulin proportion in the pancreas indicates that in the beta cells the relative proportion of immature granules [35, 36] and/or of a cytoplasmic fraction [8, 9, 37] both of which are rich in proinsulin, may be increased. It has been suggested that the cytoplasmic fraction may be released by a non-granular route, under conditions of high secretion rate [9, 37]. These pancreatic and serum changes suggest that growth hormone caused in the fasting state a preferential release of immature granules or of a cytoplasmic fraction, to produce an increase in the proportion of proinsulin in the circulation.

In the response of healthy subjects to glucose infusion, the systemic rise in insulin exceeds that in proinsulin: the effect has been attributed to accelerated secretion of IRI, similar in composition to pancreatic IRI, and to the consequent lesser influences of the concurrent differential clearances of these peptides [1, 3, 30]. This decrease in the serum proinsulin proportion in response to glucose infusion was found also in normal dogs. Growth hormone magnified the insulin response to glucose infusion, while the proinsulin proportion decreased. The result suggests that growth hormone enhanced the secretory response of the beta cells in the release of mature granules.

Metasomatotrophic diabetes is due to beta cell damage produced by prolonged over-stimulation by growth hormone and resulting in reduced synthesis and secretion of insulin [20, 38]. At one month of this diabetes, none of the dogs had received insulin treatment, serum insulin was low and the proportion of proinsulin was elevated. In the small amount of insulin remaining in the pancreas after 14 months of this diabetes, the proportion of proinsulin was high, which may be indicative of deficient conversion of proinsulin to insulin and C-peptide. The increased proportion of proinsulin in the circulation may be due to an increase in the proportion of proinsulin in the beta cell secretion. The absence of change in serum insulin and proinsulin after glucose infusion in this diabetes is indicative of inability of the beta cells to respond to this stimulus. In view of the existing hyperglycaemia, this deduction could be questioned; however, IRI release responses to other stimuli were also absent [20]. In normal dog pancreas, the concentration and amount of IRI were in the decreasing order of tail, body and head regions, in agreement with other observations [39]. In growth hormone treatment and in metasomatotrophic diabetes, the reductions in pancreatic IRI were most pronounced in the tail region.

In relation to these observations, only very brief mention can be made of the extensive studies on insulin and proinsulin in diabetes mellitus [1]. In diabetic insulinopenic patients, the increase in the proinsulin component was attributed to the secretion of immature granules containing a high proportion of proinsulin [10]. High proinsulin levels were found in maturity-onset diabetics [12, 14] and a high proportion of proinsulin was secreted by insulin-requiring diabetics [13], presumably due to defective formation of mature secretor granules by the beta cells.

In summary, the serum proinsulin proportion is increased by growth hormone treatment, in association with hypersecretion of insulin and hyperinsulinaemia. The proinsulin proportion in serum is also increased in metasomatotrophic diabetes, associated with hyposecretion of insulin and hypoinsulinaemia.

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