The gastric secretory response to a continuous insulin infusion in the dog

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SUMMARY The gastric acid response to graded doses of insulin given by continuous infusion was studied in four dogs, each surgically provided with a gastric fistula. All doses of insulin resulted in a prolonged plateau of hypoglycaemia and the degree of hypoglycaemia correlated significantly ($P < 0.05$) with the insulin dose, up to 0.15 u/kg hr. Similarly, graded doses of insulin (up to 0.15 u/kg hr) produced graded acid responses and both the peak acid output and the total acid output correlated significantly with blood glucose changes. No initial inhibitory phase of acid secretion followed the start of the infusion, but a dose-related delay in the onset of the acid response was observed. Our results indicate that insulin provides a quantitative glycopenic stimulus producing a quantitative vagal acid response.

Insulin hypoglycaemia provides a glycopenic stimulus producing a vagal gastric secretory response (Jögi, Ström, and Uvnäs, 1949). Earlier investigators (Hollander, Jemerin, and Weinstein, 1942; Davis, Brooks, and Robert, 1965) believed that the gastric acid response to insulin hypoglycaemia represented an 'all-or-none' phenomenon which was (1) initiated when the blood glucose fell to a given threshold value and (2) not related to the degree of hypoglycaemia below such a threshold. The recent finding, however, of a quantitative relationship between insulin dose, hypoglycaemia, and acid secretion (Baron, 1970; Spencer and Grossman, 1971) does not support the 'all-or-none' hypothesis.

A single rapid intravenous injection of insulin, such as used by the previously mentioned workers, produces a nadir of hypoglycaemia with gradual restoration of normoglycaemia. In human subjects (Carter, Dozois, and Kirkpatrick, 1972) an infusion of insulin provides a steady level of hypoglycaemia which, if reproducible in the dog, should facilitate correlation studies between blood glucose changes and gastric acid changes.

The present investigation was undertaken to study the effect of a continuous infusion of insulin on the blood glucose response in the dog and to establish the relationship between insulin dose, degree of hypoglycaemia, and acid response.

Methods

Four mongrel male dogs, 10 to 14 kg in weight, were each surgically provided with a chronic gastric fistula (Thomas, 1941). Following a three-week recovery period, the gastric secretory studies were started. The dogs were fasted for 20 hours before each test and stood on a Pavlov stand during the test.

After collection of two 15-minute samples of basal gastric secretion the insulin infusion was commenced. Soluble insulin (Insulin BP, Burroughs Wellcome), in doses of 0.025, 0.05, 0.15, and 0.30 units per kg of body weight per hour, was diluted in 154 mM NaCl and infused intravenously for three hours, using a constant slow injection pump (C. F. Palmer, London). Gastric juice was collected continuously for three hours and divided into 15-minute samples. The volume (ml/15 min) and acid concentration (m-equiv/l) were measured and the acid output (m-equiv/15 min) was calculated. All titrations were made to pH 7.0 with 0.01 N NaOH, using an EIL titrimeter (Electronic Instruments Limited, Richmond, Surrey, England). Samples of venous blood were also obtained just before and every 30 minutes for two and a half hours after the start of the infusion and blood glucose levels determined by a glucose oxidase method using an AutoAnalyzer.

Three tests were carried out on each dog at each dose in random order. No animal was tested more than once every 48 hours.

Terminology

The following variables of blood glucose were examined: (1) the plateau blood glucose (PBG), calculated as the mean of blood glucose values at 60, 90, 120, and 150 minutes after the start of the infusion and expressed in mg/100 ml; (2) the lowest blood glucose (LBG), being the lowest blood glucose value observed during the insulin infusion, expressed in mg/100 ml; and (3) the Δ blood glucose (Δ BG), being the difference between the initial fasting blood glucose and the plateau blood glucose, expressed as a percentage of the fasting blood glucose.

With regard to acid secretion, two variables were examined: (1) the peak acid output (PAO), calculated as the sum of the two highest consecutive 15-minute collections during the infusion, multiplied by 2 and expressed as m-equiv/hr (Baron, 1963); (2) the total acid output (TAO), calculated as the sum of all 12 15-minute samples during the insulin infusion, expressed as m-equiv/3 hr.

Results

BLOOD GLUCOSE RESPONSE

The pattern of blood glucose response to the insulin infusion was essentially the same for each dose in each dog (fig 1). By one hour, a level of hypoglycaemia was reached which remained fairly constant during the second and third hour of the infusion. There was a tendency for the lowest blood glucose value to be reached later during the infusion with

Fig 1 Pattern of blood glucose response to continuous infusion of insulin. Each point represents the overall mean of 12 observations in four dogs.

Fig 2 Blood glucose changes related to insulin dose. The Δ blood glucose ($\circ$) significantly ($p < 0.02$ or less, Student's t test for unpaired data) increased and the plateau blood glucose ($\bullet$) significantly ($p < 0.05$ or less, Student's t test for unpaired data) decreased with increasing doses of insulin, up to 0.15 u/kg hr. Each point represents the mean of 12 observations in four dogs ($\pm$ SE).

the lowest two doses of insulin (0.025 and 0.05 u/kg hr), but values obtained at 60, 90, 120, and 150 minutes were not statistically different. The PBG and the LBG did not differ significantly. The PBG decreased and the Δ BG increased significantly with increasing doses of insulin up to 0.15 u/kg hr (fig 2).

Blood glucose changes produced by the highest two doses of insulin (0.15 and 0.30 u/kg hr) were nearly identical, and accordingly the insulin doses (excluding 0.30 u/kg hr) correlated significantly with the PBG and the Δ BG (table I). These results indicate that increasing doses of insulin up to 0.15 u/kg hr

Dog	r^1	p
Plateau Blood Glucose		
A	-0.70	<0.05
B	-0.85	<0.01
C	-0.71	<0.05
D	-0.86	<0.01
Δ Blood Glucose		
A	0.85	<0.01
B	0.80	<0.01
C	0.69	<0.05
D	0.81	<0.01

Table I Linear correlations between doses of insulin (0.025, 0.05, and 0.15 u/kg hr) and blood glucose changes

¹Correlation coefficient derived from nine observations (three at each of the three doses of insulin) in each dog.

produced significantly increasing degrees of hypoglycaemia.

GASTRIC ACID RESPONSE

The mean secretory responses to the insulin infusion in all tests are shown in figure 3. Low basal outputs of acid were constantly observed and no initial inhibition of acid secretion following the start of the infusion could be detected. In each test in each of the four dogs and with every dose of insulin tested, a delay in the onset of the acid response was observed. This delay varied between dogs for each dose, but was dose-related in that progressively shorter delays were noted with increasing doses of insulin, up to 0.15 u/kg hr (fig 3).

Following this initial delay in the onset of acid secretion, the acid output rapidly increased. With the lowest dose of insulin capable of initiating acid secretion (0.025 u/kg hr for dogs B and D and 0.05 u/kg hr for dogs A and C), the output of acid tended to follow a plateau for the duration of the test. With the higher doses, a peak was reached after which the acid output declined in a stepwise fashion for the

remainder of the infusion (fig 3). This decline in acid output, following the initial peak, resulted from a fall in both the volume and the acid concentration of gastric juice. The PAO and the TAO increased significantly ($p < 0.05$ or less, Student's *t* test for unpaired data) with increasing doses of insulin up to 0.15 u/kg hr (figs 4 and 5). Doubling the dose of insulin to 0.30 u/kg hr tended to produce lower PAO and lower TAO, but this was not consistent and did not achieve statistical significance.

CORRELATION BETWEEN BLOOD GLUCOSE CHANGES AND OUTPUTS OF ACID

In two dogs (A and C) no secretion was initiated with a dose of insulin of 0.025 u/kg hr (fig 3), which produced the lowest blood glucose values ranging from 44 to 60 mg/100 ml. In dog C a PBG of 40 mg/100 ml in one test resulted in significant acid production.

A statistically significant inverse linear correlation was observed between both the PAO and the TAO and the PBG and the LBG, indicating that the outputs of acid increased with increasing degrees of

Fig 3 Output of acid to continuous insulin infusion. Each point represents the mean of three observations.

Fig 4 Peak acid output related to insulin dose. The PAO increased significantly ($p < 0.05$ or less, Student's t test for unpaired data) with increasing doses of insulin up to 0.15 u/kg. hr. Each point represents the mean of 12 observations in four dogs ($\pm$ SE). Same applies to figure 5.

Fig 5 Total acid output related to insulin dose. The TAO increased significantly ($p < 0.05$ or less, Student's t test for unpaired data) with increasing doses of insulin up to 0.15 u/kg. hr.

Dog	Acid Output	Blood Glucose					
		PBG ¹ (mg/100 ml)		LBG ¹ (mg/100 ml)		Δ BG ¹ (%)	
		r	p	r	p	r	p
A	PAO ²	-0.85	<0.001	-0.84	<0.01	+0.92	<0.001
	TAO	-0.82	<0.01	-0.80	<0.01	+0.91	<0.001
B	PAO	-0.71	<0.05	-0.64	<0.05	+0.74	<0.05
	TAO	-0.73	<0.05	-0.63	<0.05	+0.69	<0.05
C	PAO	-0.65	<0.05	-0.70	<0.05	+0.70	<0.05
	TAO	-0.60	<0.05	-0.67	<0.05	+0.55	>0.05
D	PAO	-0.77	<0.01	-0.76	<0.05	+0.77	<0.05
	TAO	-0.76	<0.01	-0.81	<0.01	+0.72	<0.05

Table II *Linear correlations between blood glucose changes and gastric acid response*¹PBG: plateau blood glucose; LBG: lowest blood glucose; Δ BG: Δ blood glucose²PAO: peak acid output; TAO: total acid output.

P < 0.05 or less: statistically significant correlation.

Fig 6 *Linear correlations between acid changes and blood glucose. The peak acid output (PAO) and the total acid output (TAO) both correlated significantly ($p < 0.05$ or less) with the plateau blood glucose (PBG), the lowest blood glucose (LBG), and the Δ blood glucose (Δ BG) in dog A (represented above) and in the other dogs (see table II).*

hypoglycaemia (table II, fig 6). The Δ BG also correlated significantly with the PAO in all dogs and with the TAO in all but one of the animals (table II).

Discussion

Our results indicate that insulin provides a quantitative glycopenic stimulus producing a quantitative vagal acid response, thus refuting the 'all-or-none' hypothesis.

In contrast to the single intravenous injection which produces a nadir of hypoglycaemia with a gradual return to normoglycaemic levels, the insulin infusion results in a prolonged 'plateau' of hypoglycaemia. The concept of an insulin infusion is not entirely new (Demand, Gross, and Berg, 1968; Carter *et al*, 1972) and we have observed a similar pattern of blood glucose response in healthy human subjects (Carter *et al*, 1972). Larger doses of insulin administered as a single injection will also tend to prolong the period of hypoglycaemia (Spencer and Grossman, 1971). In our experiments, graded doses of insulin up to 0.15 u/kg hr produced graded levels of hypoglycaemia.

Similarly, graded doses of insulin resulted in a graded acid response. Such findings have been reported in man (Baron, 1970; Isenberg, Stening, Ward, and Grossman, 1969), in the dog (Cooke, 1969; Spencer and Grossman, 1971), and in the cat (Stening and Isenberg, 1969), where insulin was administered as a single intravenous injection.

The infusion of insulin produced a steady level of hypoglycaemia, but a peak rather than a plateau of acid secretion was observed with larger doses of insulin. Such findings would indicate that the acid response, initiated by the fall in blood glucose or the nadir, is not sustained by prolongation of the period of hypoglycaemia and that this phenomenon could be dose-dependent. Persistent hypoglycaemia would appear to provide a continuous glycopenic stimulus since lower doses of insulin tended to produce a steady state of acid secretion. However, prolonged hypoglycaemia due to larger doses of insulin may be concomitantly associated with important and persistent inhibition of acid secretion. This is suggested by the observation that the largest two doses of insulin used in our experiments produced peak acid outputs of comparable magnitude while the subsequent decline in acid secretion was consistently steeper and more important with the largest dose. Work is in progress to determine if changes in potassium levels are responsible for this phenomenon. In man, blood glucose values of less than 15 mg/100 ml were associated with inhibition of gastric acid secretion (Baron, 1970). In the dog, blood 'sugar' values of less than 25 mg/100 ml have

been reported to be associated with inhibition of gastric acid secretion (Neches, Olson, and Scruggs, 1942) and of pancreatic (Scott, Collignon, Bugel, and Johnson, 1941) and intestinal secretion (Kneller and Nasset, 1949). There was, however, a definite quantitative relationship between the insulin dose, the degree of hypoglycaemia, and the magnitude of the acid response.

No initial inhibitory phase of acid secretion could be detected following the start of the insulin infusion. This is contrary to previous workers' findings (Olson and Neches, 1955) who used insulin preparations 'contaminated' with glucagon, which might have been responsible for such an effect (Aylett, 1962), but is in agreement with Spencer and Grossman's findings (1971) who used, like us, purified insulin. Insulin is by itself, however, a powerful inhibitor of histamine-(Hirschowitz and Robbins, 1966) and pentagastrin-(Hirschowitz and Sachs, 1972) induced acid secretion. In our tests, a delay in the onset of the acid response was consistently seen following the commencement of the infusion. Such delay appeared to be dose-related in that shorter delays were observed with larger doses of insulin.

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References

- Aylett, P. (1962). The effects of glucagon and glucagon-free insulin upon gastric secretion in peptic ulcer patients. *Clin. Sci.*, **22**, 179-184.
- Baron, J. H. (1963). Studies of basal and peak acid output with an augmented histamine test. *Gut*, **4**, 136-144.
- Baron, J. H. (1970). Dose response relationships of insulin hypoglycaemia and gastric acid in man. *Gut*, **11**, 826-836.
- Carter, D. C., Dozois, R. R., and Kirkpatrick, J. R. (1972). Insulin infusion test of gastric acid secretion. *Brit. med. J.*, **2**, 202-204.
- Cooke, A. R. (1969). Acid and pepsin secretion in response to endogenous and exogenous cholinergic stimulation and pentapeptide. *Aust. J. exp. Biol. med. Sci.*, **47**, 197-202.
- Davis, R. A., Brooks, F. P., and Robert, C. M., Jr. (1965). Gastric secretory response to graded insulin hypoglycaemia. *Amer. J. Physiol.*, **208**, 6-8.
- Demand, H. A., Gross, H. U., and Berg, G. (1968). Effects of continuous insulin infusions on unstimulated human gastric secretion. *Gastroenterology*, **54**, 1038-1049.
- Hirschowitz, B. I., and Robbins, R. C. (1966). Direct inhibition of gastric electrolyte secretion by insulin, independent of hypoglycemia or the vagus. *Amer. J. dig. Dis.*, **11**, 199-212.
- Hirschowitz, B. I., and Sachs, G. (1972). KCl reversal of insulin inhibition and fade in pentagastrin-stimulated gastric secretion. *Amer. J. Physiol.*, **223**, 305-309.
- Hollander, F., Jemerin, E. E., and Weinstein, V. A. (1942). An insulin test for differentiating vagal from non vagal stomach pouches. (Abstr.) *Fed. Proc.*, **1**, 116.

- Isenberg, J. I., Stening, G. F., Ward, S., and Grossman, M. I. (1969). Relation of gastric secretory response in man to dose of insulin. *Gastroenterology*, 57, 395-398.
- Jögi, P., Ström, G., and Uvnäs, B. (1949). The origin in the CNS of gastric secretory impulses induced by hypoglycemia. *Acta physiol. scand.*, 17, 212-221.
- Kneller, A. W., and Nasset, E. S. (1949). Relationship of insulin hypoglycemia to intestinal secretion. *Amer. J. Physiol.*, 159, 89-94.
- Necheles, H., Olson, W. H., and Scruggs, W. (1942). The effect of insulin on gastric secretion. (Abstr.) *Fed. Proc.*, 1, 62.
- Olson, W. H., and Necheles, H. (1955). Primary depression of gastric

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- secretion by insulin in normal man. *J. Amer. med. Ass.*, 159, 1013-1014.
- Scott, V. B., Collignon, U. J., Bugel, H. J., and Johnson, G. C. (1941). The relation of external pancreatic secretion to variations in blood sugar. *Amer. J. Physiol.*, 134, 208-218.
- Spencer, J., and Grossman, M. I. (1971). The gastric secretory response to insulin: an 'all-or-none' phenomenon? *Gut*, 12, 891-896.
- Stening, G. F., and Isenberg, J. I. (1969). Insulin-induced acid secretion after partial vagotomy in dogs and cats. *Amer. J. Physiol.*, 217, 962-964.
- Thomas, J. E. (1941). An improved cannula for gastric and intestinal fistulas. *Proc. Soc. exp. Biol. (N.Y.)*, 46, 260-261.

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