Comparative effect of human soluble insulin and insulin aspart upon hypoglycaemia-induced alterations in cardiac repolarization

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Aims Sudden death in young diabetic patients has been associated with nocturnal hypoglycaemia perhaps as a result of cardiac dysrhythmias following abnormal cardiac repolarization during hypoglycaemia. It was therefore important to compare the effect of soluble human insulin (HI) and a rapid-acting insulin analogue, insulin aspart (IAsp), on these aspects of cardiac function.

Methods A total of 17 healthy males underwent identical hyperinsulinaemic hypoglycaemic clamps with blood glucose maintained at 5 mM for 30 min and reduced to 2.5 mM after an additional 30 min. Subjects received either HI or IAsp on two different occasions separated by 4–6 weeks. Regular measurements were made of two measures of cardiac repolarization, QT dispersion and QTc as well as of counter-regulatory hormones.

Results The blood glucose lowering effect did not differ between IAsp and HI and the clearance rates were similar (HI mean ± SD 1.24 ± 0.12 l h^{-1} kg^{-1}, IAsp mean ± s.d. 1.22 ± 0.32 l h^{-1} kg^{-1}). There were similar significant increases but no difference between treatments in QTc after hypoglycaemia induced by either IAsp or HI (480 ± 37 ms *vs* 480 ± 25 ms; NS). However, QT dispersion during hypoglycaemia was less pronounced with IAsp than with HI (92 ± 36 ms *vs* 107 ± 42 ms; $P < 0.05$). Plasma adrenaline increased significantly and similarly after both insulins (initial and final concentration, HI, 0.23 ± 0.01 to 4.87 ± 0.48 nM, $P < 0.001$, IAsp, 0.24 ± 0.01 to 4.99 ± 0.48 nM, $P < 0.001$). Serum potassium decreased significantly but by a similar amount between the groups (initial and final concentration, HI, 4.18 ± 0.3 to 4.2 ± 0.2 mM, $P < 0.001$, IAsp, 4.2 ± 0.3 to 4.2 ± 0.3 mM, $P < 0.001$).

Conclusions Soluble human insulin and insulin aspart had similar effects upon hypoglycaemia-induced alterations in cardiac repolarization, presumably because the effects of both regular insulin and insulin aspart on the sympathoadrenal response and potassium concentration were the same.

Keywords: human insulin, hypoglycaemia, insulin aspart, ventricular repolarization

Introduction

The aim of insulin treatment in type I diabetes mellitus is to reproduce physiological insulin secretion profiles optimizing glycaemic control and preventing microvascular complications [1, 2]. However, the limitations of subcutaneous insulin delivery mean that even the most modern insulin regimens, produce near normoglycaemia only at the expense of a high risk of severe hypoglycaemia.

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This is partly because standard human insulin (HI) aggregates as hexameric macromolecules in neutral solution slowing its absorption following subcutaneous administration [3, 4]. Thus, patients are exposed to the risk of both postprandial hyperglycaemia and postabsorptive hypoglycaemia, particularly at night [5]. Insulin aspart (IAsp) is a novel, rapid-acting, monomeric insulin analogue in which the amino acid proline, at position 28 on the B chain, is replaced by aspartic acid. IAsp was designed to mimic HI in biological aspects, but with a faster absorption, earlier onset and shorter duration of action, characteristics confirmed in several trials in healthy subjects and type I diabetic patients [6–9]. In large clinical trials the use of IAsp results in minor but significantly improved glycaemic control compared with

HI without increasing the incidence of hypoglycaemia [10].

Young people with type 1 diabetes may be at risk of sudden death from hypoglycaemia particularly at night (the 'dead in bed' syndrome) [11]. The initial suggestion that this was due to the introduction of human insulin has been largely refuted [12] and there is increasing evidence to support an alternative explanation. Insulin-induced hypoglycaemia causes a fall in potassium that in combination with sympathoadrenal activation leads to abnormalities of cardiac repolarization [13] manifested by lengthening of the QT interval in patients with both type 1 and type 2 diabetes [14]. A prolonged QT interval is a predictor of malignant cardiac dysrhythmias and sudden death in conditions including the acute phase of myocardial infarction and congenital long QT syndrome [15, 16]. Other factors which might influence the risk of sudden death in those with diabetes such as autonomic neuropathy are currently being investigated [17].

In view of this potentially pathogenetic side-effect of insulin and the controversy around sudden death which followed the introduction of human insulin, we thought it important to establish whether novel analogues exhibit similar properties. Therefore the aim of our study was to compare the effect of experimental hypoglycaemia induced by HI and IAsp upon markers of cardiac repolarization. We hypothesized that as both insulin types caused similar sympathoadrenal responses to hypoglycaemia the effect on cardiac electrophysiological responses would also be comparable.

Methods

Subjects

We recruited 17 healthy white Caucasian males aged 18–40 years with a normal electrocardiograph (ECG), and no signs of previous cardiac events. Further inclusion criteria included body mass index of ≤ 27 kg m^{-2}, non-smoking history for at least the previous 3 months and good general physical health, based on medical history, physical examination and laboratory assessments. Subjects provided written informed consent prior to entering the study which was approved by the Northern General Hospital Ethics Committee.

Study design

Subjects were randomized to receive either IAsp or HI on two separate occasions, separated by 28–56 days. During each of the trial days, following 30 min rest, subjects received an infusion of IAsp or HI for a total of 120 min. After 10 min, the infusion rate was fixed at 1.5 mU kg^{-1} min^{-1} so that subjects received a minimum of 120 mU kg^{-1} of IAsp or HI. Blood glucose was measured at 2–5 min intervals at the bedside using a glucose oxidase method (YSI3500, YSI Inc., Yellow Springs, Ohio, USA) and controlled throughout the procedure by adjusting the rate of a 20% dextrose infusion. Blood glucose was clamped at 5 mmol l^{-1} for the first 30 min, then reduced to 4 mmol l^{-1} over 30 min, further reduced to 2.5 mmol l^{-1} over the next 30 min, at which it was clamped for a final 30 min. Plasma samples were obtained at 0, 30, 40, 50, 60 90, 100, 110 and 120 min for later measurement of potassium, magnesium, calcium, glucagon, catecholamines, insulin and C-peptide.

Measures of cardiac repolarization

ECGs were recorded on a custom built system for high-resolution ECG analysis that simultaneously acquires three bipolar orthogonal X, Y and Z leads [14]. An average ECG profile was derived from each recording period of approximately 6 min. Signals from sinus beats for each lead were amplified (5000×0.05–250Hz), digitized (5 μV resolution at 750 samples s^{-1}) and filtered on line using a 50 Hz linear recursive filter. Signal averaging was performed by using an initial beat as the template for a template-matching scheme [18]. A single beat from every 6 s interval was accepted if it had a correlation coefficient with the template greater than 99.5%. Accepted beats were averaged until recording was manually terminated when the mean residual noise was below 2 μV. Typically, averaging of 10–30 beats was required to achieve this figure. If fewer than 10 beats were averaged, an infrequent occurrence resulting from poor template selection, the recording was manually rejected and a new recording initiated. The associated mean heart rate over the recording period was also recorded.

Digitized ECGs were taken continuously during the 120 min procedure, and a 12-lead ECG was performed at 0, 30, 60, 90 and 120 min. Pulse and blood pressure were recorded every 10 min.

QT interval

The QT interval was measured in the averaged X,Y and Z signals individually. QT interval was measured using a tangent drawn at the point of maximal gradient on the downward slope of the T wave, and the end of the T wave marked as the point of intersection of the tangent with the isoelectric line [19]. The QT intervals were marked using an on-screen cursor (Figure 1) by two independent observers, blinded both to the prevailing blood glucose and to the outcome of previous measurements and calculated as the mean of the two measurements. The X, Y and Z readings were then averaged to obtain an overall measure of the repolarization process.

We corrected for differences in heart rate, using the Bazett correction which mathematically normalizes the QT interval to a heart rate of 60 beats min^{-1} [15].

QT dispersion

Twelve-lead ECGs were recorded on a Burdick electrocardiograph at a paper speed of 50 mm s^{-1}, and enlarged two-fold prior to analysis. Each ECG was analysed manually by two blinded observers and the mean QT dispersion recorded. QT dispersion was defined as the difference between the maximum and minimum QT across the 12-lead ECG [20].

Metabolic measurements

Plasma adrenaline and noradrenaline were analysed by high-performance liquid chromatography (h.p.l.c.) using electrochemical detection [21], and plasma C-peptide and glucagon by standard radioimmunoassays [22, 23]. Insulin was analysed using a radioimmunoassay (Pharmacia RIA 100, Uppsala, Sweden, validated for insulin aspart [24]). Serum potassium was analysed by direct potentiometry (Vitros Analyzer, Johnson & Johnson Orthoclinical Diagnostics, Haversham, Bucks, UK).

Statistical analysis

Our sample size of 16 was chosen on the basis that this would have 90% power to demonstrate comparable effects of IAsp and HI on the change in QTc interval (a difference of 19 ms) and QT dispersion (a difference of 9 ms) during hypoglycaemia, assuming a coefficient of variance of 15% [25].

Data are expressed as mean ± SD or 95% CI. We used unpaired *t*-tests to compare responses between groups and paired tests to compare responses within groups, using the summary measures of basal and maximal responses during euglycaemia and hypoglycaemia. A P value of < 0.05 was considered significant.

Results

All 17 recruited subjects completed the study. They were healthy male Caucasians with a mean age of 28.0 ± 6.4 years and with a body mass index of 23.8 ± 1.9 kg m^{-2}.

The two insulins performed identically and produced nondistinguishable reduction in glucose during i.v. infusion. Mean insulin clearance values were similar for both treatments: 1.22 ± 0.32 l h^{-1} kg^{-1} for IAsp and 1.24 ± 0.12 l h^{-1} kg^{-1} for HI.

Infusion rates of 20% glucose were similar for both insulin species. During euglycaemia, the mean glucose infusion rate for HI was 168 ± 69 ml h^{-1} and for IAsp 162 ± 79 ml h^{-1} [difference 6 ml h^{-1} (-3, 15, $P = 0.22$)]. During hypoglycaemia mean glucose infusion rate for HI was 93 ± 59 ml h^{-1} and for IAsp 89 ± 58 ml h^{-1} [difference 4 ml h^{-1} (-7, 15, $P = 0.44$)].

Measures of cardiac repolarization

QTc (Table 1) During euglycaemia, QTc increased significantly by 15 ms (95% CI 9, 22, $P < 0.0001$) with HI and by 21 ms (13, 28, $P < 0.0001$) with IAsp, although the difference between insulin species was not significant (–6, –12.1, 0.58, $P = 0.07$). During hypoglycaemia, QTc increased (from baseline) by 89 ms (78; 100, $P < 0.0001$) with HI and 90 ms (72, 108, $P < 0.0001$) with IAsp but there was no difference between insulin species (–16.7, 14.1, $P = 0.857$).

QT dispersion During euglycaemia QT dispersion increased from baseline significantly by 10 ms (1, 16, $P = 0.002$) with HI, but was unchanged with IAsp (by

Table 1 Measures of cardiac repolarization at baseline, during euglycaemia and during hypoglycaemia.

	HI		*IAsp*		*Difference HI-IAsp*	
Parameter	*Mean*	*SD*	*Mean*	*SD*	*95% CI*	P *value*
QTc (ms)						
Baseline	390	17	391	22	–9.9, 11	0.93
Euglycaemia	406	18	411	22	–13.5, 3.2	0.30
Hypoglycaemia	480	259	480	37	–18.3, 16.8	0.95
Change baseline hypoglycaemia	89[a]	21	90[a]	35	–16.7, 14.1	0.86
QT dispersion (ms)						
Baseline	40	7	45	11	–11.6, 1.34	0.11
Euglycaemia	50	11	50	16	–9.1, 8.1	0.98
Hypoglycaemia	107	42	92	36	1.65, 28.5	0.03
Change baseline hypoglycaemia	67[a]	42	47[a]	32	6.93, 33.4	0.005

[a]Indicates significant difference from baseline.

Table 2 Metabolic measurements at baseline, during euglycaemia and during hypoglycaemia.

	HI		*IA*		*Difference HI-IAsp*	
Parameter	*Mean*	*SD*	*spmean*	*SD*	*95% CI*	P *value*
Potassium (mM)						
Baseline	4.18	0.28	4.15	0.25	−0.12, 0.17	0.73
Euglycaemia	3.75	0.18	3.66	0.15	−0.02, 0.20	0.12
Hypoglycaemia	3.42	0.22	3.35	0.21	−0.05, 0.19	0.23
Change baseline hypoglycaemia	0.76[a]	0.28	0.81[a]	0.27	−0.2, 0.11	0.52
Adrenaline (nM)						
Baseline	0.23	0.10	0.24	0.09	−0.06, 0.04	0.66
Euglycaemia	0.26	0.09	0.23	0.09	−0.04, 0.10	0.38
Hypoglycaemia	4.87	1.99	4.99	2.09	−1.11, 0.87	0.80
Change baseline hypoglycaemia	4.68[a]	1.98	4.81[a]	2.05	−1.11, 0.85	0.79
Noradrenaline (nM)						
Baseline	1.28	0.45	1.37	0.45	−0.38, 0.21	0.53
Euglycaemia	1.59	0.57	1.55	0.49	−0.36, 0.45	0.83
Hypoglycaemia	2.21	0.79	2.14	0.72	−0.43, 0.56	0.78
Change baseline hypoglycaemia	0.92[a]	0.52	0.77	0.44	−0.18, 0.49	0.34
Glucagon (pM)						
Baseline	33.9	5.32	33.6	3.83	−2.71, 3.42	0.81
Euglycaemia	30.8	5.30	30.5	3.61	−2.62, 3.21	0.83
Hypoglycaemia	44.2	5.78	42.8	7.31	−1.98, 4.80	0.39
Change baseline hypoglycaemia	10.29[a]	5.34	9.24[a]	5.38	−1.91, 4.03	0.46

[a]Indicates significant difference from baseline.

5 ms 0, 10, $P = 0.066$) although there was no significant difference between insulin species (−1.55, 11.5, $P = 0.125$). During hypoglycaemia, QT dispersion increased by 67 ms (45, 88, $P < 0.0001$) with HI and 47 ms (30, 63, $P < 0.0001$) with IAsp and the increase observed with HI was significantly greater compared with IAsp (6.93; 33.4, $P = 0.005$).

Potassium (Table 2)

Potassium fell significantly from baseline during clamped euglycaemia with both insulins but there was no difference between the two groups. The falls during hypoglycaemia were also significant but no different between the insulin species.

Adrenaline (Table 2)

Adrenaline concentrations were similar at baseline and remained unchanged from baseline for either insulin during euglycaemia. During hypoglycaemia, adrenaline increased significantly and to a comparable degree with both insulin species.

Noradrenaline (Table 2)

Noradrenaline concentrations were similar at baseline. During euglycaemia with HI and IAsp, noradrenaline concentrations increased slightly but significantly with both insulins although there was no significant difference between insulin species. During hypoglycaemia, noradrenaline increased significantly (from baseline) and to a comparable degree with both insulins.

Glucagon (Table 2)

Glucagon concentrations were similar at baseline. During euglycaemia there was a significant but comparable fall in glucagon with both insulins. During hypoglycaemia glucagon concentrations increased significantly (from baseline) but there was no difference between insulin species.

Calcium and magnesium remained unchanged throughout the study (data not shown).

Discussion

These data show that experimental hyperinsulinaemic hypoglycaemia induced in nondiabetic subjects produces abnormal cardiac repolarization with lengthening of both QTc and QT dispersion. The increases were both statistically and clinically significant, producing mean rises in QT interval of around 90 ms and including some subjects whose QTc reached maximum values above 500 ms. These changes are associated with malignant arrhythmias in other conditions such as acute myocardial infarction. QT dispersion also increased indicating that insulin-

induced hypoglycaemia prolongs other indices of cardiac repolarization.

There was no significant difference in increases in QTc whether hypoglycaemia was induced by soluble insulin or IAsp. This suggests that the effect of insulin-induced hypoglycaemia on this measure of cardiac repolarization was unrelated to insulin species and is a consequence of either hypoglycaemia itself, the potassium lowering effect of insulin or the sympathoadrenal discharge induced by hypoglycaemia.

HI and IAsp exhibit similar binding to the insulin receptor in *in vitro* studies [26]. There is considerable evidence that the two preparations have equivalent metabolic effects. It is therefore unsurprising that when infused intravenously, both preparations elicited similar falls in serum potassium. Previous work has demonstrated comparable counter-regulatory and symptomatic responses during hypoglycaemia [27] and we confirmed that rises in adrenaline (and glucagon) were no different during hypoglycaemia induced by either insulin.

Cardiac rhythm disturbances have been described during clinical episodes of hypoglycaemia in patients with type 1 diabetes, including atrial fibrillation [28] and ventricular ectopic activity [29]. Experimental hypoglycaemia alters ventricular repolarization with prolongation of the QT interval [14], indicating a possible mechanism which might explain the increased risk of sudden overnight deaths in young type 1 diabetic patients [11]. In the early 1990s, it was claimed that these deaths were linked to the use of human insulin [30, 31], but a recent systematic review found no evidence that HI has a specific effect on the physiological response to hypoglycaemia when compared with insulin of other species [12]. Nevertheless, as a range of medications, including anti-arrhythmic drugs, antihistamines and antibiotics can prolong cardiac repolarization [32], the controversy surrounding HI highlights the importance of assessing the effect of new insulin analogues on the electrocardiogram.

Unexpectedly, we found a significant difference in maximum increases in QT dispersion between insulin species with increased intervals observed during human insulin infusion. This might reflect differing actions of each insulin species on this measure of ventricular repolarization and support the contention that some types are safer than others. It seems more likely that it represents a statistical quirk, particularly as the mean difference was small and not observed in other measures of repolarization. QT dispersion is difficult to measure consistently with interobserver variability in QT-dispersion measurements rising as high as 30%, particularly if ECG morphology is abnormal [33]. There is a clear relation between QT measurement error and ST-T amplitude [34] and during hypoglycaemia, ECG morphology alters, with flattening of the ST segment. Thus, although we believe this observation may be worthy of further investigation, its clinical relevance seems doubtful.

Our experimental model, the hyperinsulinaemic clamp, provides consistent and equivalent hypoglycaemic stimuli with which to compare physiological responses [35]. Among its limitations is the need to use high concentrations of intravenous insulin (100–200 mU l^{-1}), which probably lower serum potassium below the concentrations experienced during subcutaneous therapy. If a low serum potassium contributes to prolonged cardiac repolarization during hypoglycaemia, then increases in QT interval and dispersion may be less marked during hypoglycaemia provoked by treatment. However, recent work suggests that sympathoadrenal activation is probably the main determinant of abnormal cardiac repolarization during hypoglycaemia [36].

These studies were conducted in normal subjects and we cannot be certain that we would have made similar observations in subjects with diabetes. However, as we have previously demonstrated comparable effects upon the electrocardiogram in studies using HI in both patients with type 1 and type 2 diabetes it is reasonable to believe that the results are clinically relevant.

In conclusion we have confirmed that experimental hypoglycaemia induced by insulin in normal subjects prolongs ventricular repolarization as measured by QTc and QT dispersion. HI and IAsp produce equivalent falls in potassium, and increases in indices of sympathoadrenal activation at comparable levels of hypoglycaemia. Both preparations had an equivalent effect on QTc interval but during hypoglycaemia QT dispersion was slightly longer during that induced with HI. The relevance of these data in an experimental model now needs to be tested by extending studies to the clinical situation.

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