DOI: 10.1111/dme.13650

Research: Pathophysiology

Salbutamol-induced electrophysiological changes show no correlation with electrophysiological changes during hyperinsulinaemic–hypoglycaemic clamp in young people with Type 1 diabetes

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Accepted 16 April 2018

Abstract

Aims Hypoglycaemia causes QT-interval prolongation and appears pro-arrhythmogenic. Salbutamol, a β_2-adrenoreceptor agonist also causes QT-interval prolongation. We hypothesized that the magnitude of electrophysiological changes induced by salbutamol and hypoglycaemia might relate to each other and that salbutamol could be used as a non-invasive screening tool for predicting an individual's electrophysiological response to hypoglycaemia.

Methods Eighteen individuals with Type 1 diabetes were administered 2.5 mg of nebulized salbutamol. Participants then underwent a hyperinsulinaemic–hypoglycaemic clamp (2.5 mmol/l for 1 h). During both experiments, heart rate and serum potassium (and catecholamines during the clamp) were measured and a high-resolution electrocardiogram (ECG) was recorded at pre-set time points. Cardiac repolarization was measured by QT-interval duration adjusted for heart rate (QT_c), T-wave amplitude (T_{amp}), T-peak to T-end interval duration (T_pT_{end}) and T-wave area symmetry (T_{sym}). The maximum changes vs. baseline in both experiments were assessed for their linear dependence.

Results Salbutamol administration caused QT_c and T_pT_{end} prolongation and a decrease in T_{amp} and T_{sym}. Hypoglycaemia caused increased plasma catecholamines, hypokalaemia, QT_c and T_pT_{end} prolongation, and a decrease in T_{amp} and T_{sym}. No significant correlations were found between maximum changes in QT_c [$r = 0.15$, 95% confidence interval (95% CI) −0.341 to 0.576; $P = 0.553$), T_pT_{end} ($r = 0.075$, 95% CI −0.406 to 0.524; $P = 0.767$), T_{sym} ($r = 0.355$, 95% CI −0.132 to 0.706; $P = 0.149$) or T_{amp} ($r = 0.148$, 95% CI −0.347 to 0.572; $P = 0.558$) in either experiment.

Conclusions Both hypoglycaemia and salbutamol caused pro-arrhythmogenic electrophysiological changes in people with Type 1 diabetes but were not related in any given individual. Salbutamol does not appear useful in assessing an individual's electrophysiological response to hypoglycaemia.

Diabet. Med. 35, 1264–1272 (2018)

Introduction

Considerable evidence indicates that hypoglycaemia is pro-arrhythmogenic during both experimental [1,2] and clinical conditions [3,4]. Hypoglycaemia causes QT_c prolongation together with pro-arrhythmogenic changes in rate-independent T-wave characteristics (T_pT_{end}, T_{sym}) via catecholamine release, hypokalaemia and inhibition of rapid delayed rectifier potassium channels (I_{Kr}) [5]. Cases of bradycardia, atrial fibrillation, atrial flutter or ventricular ectopic beats linked with hypoglycaemia have been reported previously [3,4,6,7]. Although hypoglycaemia continues to be very common in people with Type 1 diabetes [8], sudden nocturnal deaths ('dead-in-bed' syndrome) [9], in which hypoglycaemia-induced malignant arrhythmias have been implicated, are fortunately very rare [10]. This indicates that the risk of arrhythmias is confined to only a few individuals

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What's new?

- We explored β_2-agonist salbutamol inhalation to identify those at risk of abnormal cardiac repolarization during hypoglycaemia. We describe the electrophysiological effects of nebulized salbutamol and a hypoglycaemic clamp in people with Type 1 diabetes.
- We confirm that both salbutamol and hypoglycaemia have pro-arrhythmogenic electrophysiological effects.
- The magnitude of electrophysiological changes induced by these two stimuli did not show any relationship as measured by a statistically significant correlation in any of the examined variables.
- An individual's electrophysiological response to inhaled salbutamol does not appear useful in predicting their electrophysiological response to hypoglycaemia.

although whether increased susceptibility is related to genetic factors, autonomic dysfunction other unrecognized influences remains unclear. If those at greatest risk of QT_c lengthening and potentially hypoglycaemia-induced arrhythmias could be identified with a simple screening test, it might then be possible to use therapeutic or technological interventions to prevent the development of malignant cardiac arrhythmias.

Salbutamol is a β-adrenoreceptor agonist with predominant β_2 activity commonly used for the treatment of asthma and chronic obstructive pulmonary disease (COPD) [11]. Salbutamol's positive chronotropic effect has been classically linked with β_1-adrenoreceptor stimulation, whereas QT_c-prolonging and hypokalaemic effects are mediated by β_2-adrenoreceptor activity [12]. We have previously shown that elevated catecholamine levels are the predominant cause of QT_c prolongation during hypoglycaemia [13]. Furthermore, pretreatment with a selective β_1-adrenoreceptor blocker atenolol [11] resulted in significant blunting of the QT_c prolongation during experimental hypoglycaemia in people with Type 1 diabetes [14]. We therefore hypothesized that establishing electrophysiological changes induced by salbutamol inhalation might offer a practical, non-invasive approach to predicting an individual's electrophysiological responses induced by hypoglycaemia. This raised the possibility of identifying high-risk candidates who might be suitable for potential prophylactic treatment with β-adrenoreceptor blockers or an implantable cardioverter defibrillator. The aim of this study was to examine the potential use of inhaled salbutamol as a screening test for those at greatest risk of abnormal cardiac repolarization during hypoglycaemia.

Participants and methods

Eighteen adults with Type 1 diabetes mellitus (age ≤ 50 years) were recruited from local diabetes outpatient clinics. Exclusion criteria were presence of ischaemic heart disease, cerebrovascular or peripheral vascular disease, asthma or COPD, presence of severe microvascular diabetic complications (diabetic maculopathy with severe visual impairment or eGFR CKD-EPI < 30 ml/min/1.73 m^2) and medication with β-blocking or QT-interval prolonging agents. Written informed consent was obtained from all participants and the study received local ethics approval.

Baseline assessment and salbutamol challenge

Each potential participant had a 12-lead electrocardiogram (ECG) performed and individuals with branch block or atrial fibrillation were excluded. Arterial BP, BMI, HbA_{1c}, urea and electrolytes, total cholesterol and triglyceride levels were obtained during the initial study visit. Cardiovascular autonomic tests were performed [15,16] to establish cardiovascular autonomic neuropathy status [17]. Participants were instructed to avoid vigorous exercise, caffeine and smoking 24 h prior to morning testing. Capillary blood glucose (BG) was measured immediately prior to administration of salbutamol to ensure participants were not hypoglycaemic (BG ≤ 3.5 mmol/l). Subsequently, 2.5 mg salbutamol was administered via a face mask. BP, heart rate, serum potassium and 5-min high-resolution three-lead ECG were measured at baseline and 10, 20, 40 and 60 min after salbutamol administration.

Hyperinsulinaemic–hypoglycaemic clamp

Following the salbutamol challenge, participants underwent a hyperinsulinaemic–hypoglycaemic clamp. Both study visits took place at least 4 weeks apart. Participants were instructed to measure BG before meals, on going to bed, at 03:00 and on waking on the day of the clamp to ensure no hypoglycaemia (BG ≤ 3.5 mmol/l) occurred 24 h prior to the clamp. Upon arrival at 08:30, an intravenous cannula was inserted into the antecubital fossa of the dominant arm initially for insulin and NaCl, and later for insulin and dextrose infusions. An infusion of 0.9% NaCl with KCl (20 mmol KCl for each 500ml of 0.9% NaCl) was initiated at a rate of 125 ml/h. For the following 4 h, BG was stabilized in the euglycaemic range (5–10 mmol/l) using a variable intravenous insulin infusion (Actrapid, Novo Nordisk Ltd, Crawley, UK) adjusted based on BG readings every 30 min. At 12:00 a retrograde cannula was inserted in the non-dominant hand in a warming chamber at 55 °C for arterialized BG, potassium and catecholamine sampling. A hypoglycaemic clamp procedure began at 13:00. A primed continuous intravenous insulin infusion was administered at 60 mU/m^2/min along with 20% dextrose commenced 4 min later at a variable rate, adjusted according to arterialized BG concentrations measured every 5 min (Analox GM9D Glucose Direct Analyser, Analox Instruments Ltd., Stourbridge, UK) for the whole duration of the clamp. Participants were blinded to BG values. From the start of the clamp, BG was

maintained at 5 mmol/l for 60 min (time points 30 EU1 to 60 EU2), then was lowered to 2.5 mmol/l over the following 30 min (until the 90 EU/HYPO time point) and was maintained at 2.5 mmol/l for 60 min (time points 90 EU/HYPO, 120 HYPO1 and 150 HYPO2). Blood pressure, heart rate and high-resolution three-lead ECG were recorded and blood samples for potassium and catecholamine levels were taken at baseline, 30 EU1, 60 EU2, 90 EU/HYPO, 120 HYPO1 and 150 HYPO2 time points as well as 30 min after the end of the procedure (180 Recov. time point). To measure catecholamines, 6 ml of whole blood was collected into chilled lithium heparin tubes containing 50 µl EGTA/glutathione preservative and centrifuged at 4 °C, 3000 rpm for 10 min. The resulting supernatant was stored at −80 °C until assayed by high-performance liquid chromatography.

ECG and cardiac repolarization

Three orthogonal ECG leads (X, Y and Z) were low-pass filtered (40 Hz) and the isoelectric line was subtracted. For each lead, an average beat was calculated from a 5-min recording. A composite wave was then calculated from averaged beats as the square root of $X^2 + Y^2 + Z^2$ and was used for further analysis of cardiac repolarization. Semiautomatic custom-built software was used to detect the fiducial points and to calculate the parameters of T-wave morphology. Measurement of the QT-interval was based on the tangent method and Bazett correction for heart rate was applied (QT_c). T-wave morphology was characterized by the T-wave amplitude (T_{amp}), normalized to the baseline [18], the T-peak to T-end interval duration (T_pT_{end}), T_pT_{end} corrected for heart rate (T_pT_{end}) and T-wave area symmetry ratio (T_{sym}), which was defined as the area under the T-wave from T-wave onset to T-peak divided by the area under the T wave between T-peak to T-end [19]. The onset and offset of the T-wave were characterized by tangents at the ascending and descending sections of the T-wave.

Statistical analysis

Statistical analysis was performed with SPSS (version 23; IBM, Chicago, IL, USA). Graphing was completed using GraphPad Prism (version 7.03, GraphPad Software, Inc., San Diego, CA, USA). Repeated measures analysis of variance (ANOVA) was applied to both salbutamol and clamp studies. The Greenhouse–Geisser correction was used to test the effect of time where the sphericity condition was violated according to Mauchly's test. Where a significant effect of time was identified, contrasts to baseline were used to compare means of variables at multiple time points during salbutamol challenge (T10, T20, T40 and T60), and euglycaemic time point (60 EU1) and hypoglycaemic time point (150 HYPO2) of the hypoglycaemic clamp procedure vs. their corresponding baseline. For all parameters, normality of residuals was confirmed by revision of QQ plots. Linear dependence between variables was examined using Pearson correlation coefficient (r). $P < 0.05$ was deemed statistically significant and correlations of > 0.6 were considered clinically relevant.

Results

Baseline characteristics and salbutamol challenge

Baseline participant characteristics are shown in Table 1. Following administration of 2.5 mg nebulized salbutamol, systolic and diastolic BP readings did not change significantly (Table 2). Serum potassium concentrations showed a non-significant trend towards lower values but remained within the normal range (Fig. 1a). A trend towards higher heart rates compared with baseline was observed from T20 onwards but did not increase significantly ($P = 0.170$ for the group) (Fig. 1b). Both QT_c interval and T_pT_{end} significantly prolonged following salbutamol inhalation ($P < 0.001$ for the group). QT_c interval was significantly prolonged at all time points vs. baseline with a maximum mean difference of 18.0 ms at T20 [95% confidence interval (95% CI) 12.5 to 23.4] (Fig. 1c). Similarly, T_pT_{end} interval duration was significantly prolonged at all time points vs. baseline with a maximum mean difference of 7.4 ms at T20 (95% CI 4.5 to 10.3) (Fig. 1d). T-wave area symmetry (T_{sym}) decreased ($P = 0.010$ for the group) after salbutamol administration. This difference was statistically significant at all time points with a maximum mean difference vs. baseline −0.082 at T20 (95% CI −0.127 to −0.038) (Fig. 1e). The normalized T-wave amplitude (T_{amp}) dropped during the salbutamol

Table 1 Baseline participant characteristics

Number of participants, *n*	18
Age (years)	35 ± 7
Male, *n* (%)	12 (66.7)
Duration of diabetes (years)	18.2 ± 7.5
BMI (kg/m^2)	26.1 ± 4.3
HbA_{1c} (mmol/mol)	68 ± 9
HbA_{1c} (%)	8.4 ± 0.8
Systolic BP (mmHg)	123 ± 12
Diastolic BP (mmHg)	74 ± 7
Heart rate (bpm)	69 ± 11
Baseline QTc (ms)	390 ± 26
Sodium (mmol/l)	137 ± 2.4
Potassium (mmol/l)	4.11 ± 0.23
Creatinine (µmol/l)	71 ± 14.0
Urea (mmol/l)	4.5 ± 1.6
Total cholesterol (mmol/l)	4.7 ± 0.6
Triglycerides (mmol/l)	1.2 ± 0.6
CAN status	
No CAN, *n* (%)	15/18 (83.3)
Possible CAN, *n* (%)	3/18 (16.7)
Definite CAN, *n* (%)	0/18 (0)

Data are displayed as mean ± SD.
CAN, cardiovascular autonomic neuropathy.

Table 2 Numerical values for examined variables during salbutamol challenge and during hyperinsulinaemic–hypoglycaemic clamp

	Salbutamol challenge					Hyperinsulinaemic–hypoglycaemic clamp		
$n = 18$	Baseline	T10	T20	T40	T60	Baseline	EU	HYPO
Systolic BP (mmHg)	122 ± 12	121 ± 10	122 ± 10	121 ± 9	122 ± 10	117 ± 15	116 ± 14	118 ± 17
Diastolic BP (mmHg)	74 ± 7	72 ± 9	72 ± 6	72 ± 6	73 ± 7	72 ± 10	74 ± 10	67 ± 11
Heart rate (bpm)	69 ± 11	70 ± 8	71 ± 12	71 ± 12	71 ± 12	67 ± 12	71 ± 12	73 ± 10
Potassium (mmol/l) ($n = 13$)†	4.04 ± 0.21	4.03 ± 0.32	4.01 ± 0.32	4.02 ± 0.28	4.02 ± 0.29	3.85 ± 0.24	3.42 ± 0.15***	3.01 ± 0.23***
QT_c (ms)	390 ± 26	405 ± 33**	408 ± 28***	405 ± 27***	405 ± 29***	403 ± 25	422 ± 27***	459 ± 34***
T_pT_{end} (ms)	67.3 ± 9.6	74.7 ± 14.3***	74.3 ± 13.5***	73.3 ± 12.3***	72.4 ± 10.5***	71.5 ± 8.1	84.4 ± 12.6***	108.6 ± 25.8***
$T_pT_{end}C$ (ms)	71.6 ± 10.8	80.3 ± 16.4***	80.5 ± 14.4***	79.2 ± 13.1***	78.3 ± 11.7***	75.1 ± 7.1	91.1 ± 14.7***	119.9 ± 31.4***
T_{amp}	1.0	0.88 ± 0.09***	0.88 ± 0.10***	0.89 ± 0.10***	0.88 ± 0.09***	1.0	0.75 ± 0.08***	0.57 ± 0.11***
T_{sym}	1.46 ± 0.23	1.39 ± 0.24*	1.38 ± 0.26**	1.38 ± 0.24**	1.40 ± 0.23**	1.35 ± 0.16	1.19 ± 0.15**	0.96 ± 0.19***
Adrenaline (nmol/l)	–	–	–	–	–	0.37 ± 0.20	0.38 ± 0.18	3.04 ± 1.59***
Noradrenaline (nmol/l)	–	–	–	–	–	1.16 ± 0.29	1.20 ± 0.32	1.85 ± 0.65***

Data are displayed as mean ± SD.
†Plasma potassium was measured in 13 participants only. Repeated measures ANOVA was used in both experiments, followed by contrasts vs the corresponding baseline. Greenhouse–Geisser correction was used in case of violated assumption of sphericity.
EU, euglycaemia, 60 min after the start of the protocol (60 EU1 timepoint); HYPO, hypoglycaemia, 150 min after the start of the protocol (150 HYPO2 timepoint).
Statistically significant changes vs. corresponding baseline: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

challenge ($P < 0.001$ for the group). The difference was statistically significant at all time points with a maximum mean difference vs. baseline −0.127 at T40 (95% CI −0.177 to −0.078) (Fig. 1f). Numerical values for all observed variables during salbutamol challenge are listed in Table 2.

Hyperinsulinaemic–hypoglycaemic clamp

Systolic and diastolic BP readings did not differ significantly between baseline, euglycaemia and hypoglycaemia (Table 2). Blood glucose levels during the clamp are shown in Fig. 2 (a). Adrenaline levels increased significantly during the hypoglycaemic part of the clamp (Δ vs. baseline 2.67 nmol/l, 95% CI 1.89 to 3.45; $P < 0.001$) but did not differ between baseline and euglycaemia (Fig. 2b). Serum potassium levels dropped significantly during the euglycaemic part of the clamp (Δ−0.43 mmol/l, 95% CI −0.50 to −0.37; $P < 0.001$) and decreased further at hypoglycaemia150 HYPO2 (Δ−0.81 mmol/l, 95% CI −1.02 to −0.59, p<0.001) (Fig. 2c). Heart rate increased gradually during the clamp, but the difference was not significant for the group ($P = 0.069$) (Fig. 2d). QT_c interval was significantly prolonged at euglycaemia (Δ19.1 ms, 95% CI 13.8 to 24.4; $P < 0.001$) and further prolonged at hypoglycaemia (Δ56.9 ms, 95% CI 43.2 to 70.5; $P < 0.001$) (Fig. 2e). Similarly, T_pT_{end} interval was prolonged at euglycaemia (Δ12.7 ms, 95% CI 8.4 to 17.0; $P < 0.001$) and further prolonged at hypoglycaemia (Δ37.1 ms, 95% CI 24.8 to 49.5; $P < 0.001$) (Fig. 2f). T-wave amplitude dropped to 75% of the baseline value at euglycaemia (Δ−0.243, 95% CI −0.282 to −0.204; $P < 0.001$) and dropped further to 57% at hypoglycaemia (Δ−0.433, 95% CI −0.492 to −0.373; $P < 0.001$) (Fig. 2g). T-wave area symmetry decreased progressively and was significantly lower vs. baseline at both euglycaemia (Δ−0.167, 95% CI −0.240 to −0.095, $P = 0.001$) and hypoglycaemia (Δ−0.390, 95% CI −0.534 to −0.246; $P < 0.001$). The T-waves became symmetric ($T_{sym} \sim 1$) at hypoglycaemia (Fig. 2h). Numerical values for all observed variables during hyperinsulinaemic–hypoglycaemic clamp are listed in Table 2.

Correlations between responses to salbutamol inhalation and to hyperinsulinaemic–hypoglycaemic clamp

We examined whether the magnitude of salbutamol-induced changes in cardiac repolarization correlated with changes induced during the hyperinsulinaemic-hypoglycaemic clamp. For this purpose, the maximum change in each parameter vs. baseline was calculated for the salbutamol challenge and for the hypoglycaemic part of the clamp. The maximum differences in both challenges were then assessed for their linear dependence. There was no statistically significant correlation between the magnitude of the QT_c interval change during salbutamol challenge and during hyperinsulinaemic–hypoglycaemic clamp ($r = 0.15$, 95% CI −0.341 to 0.576;

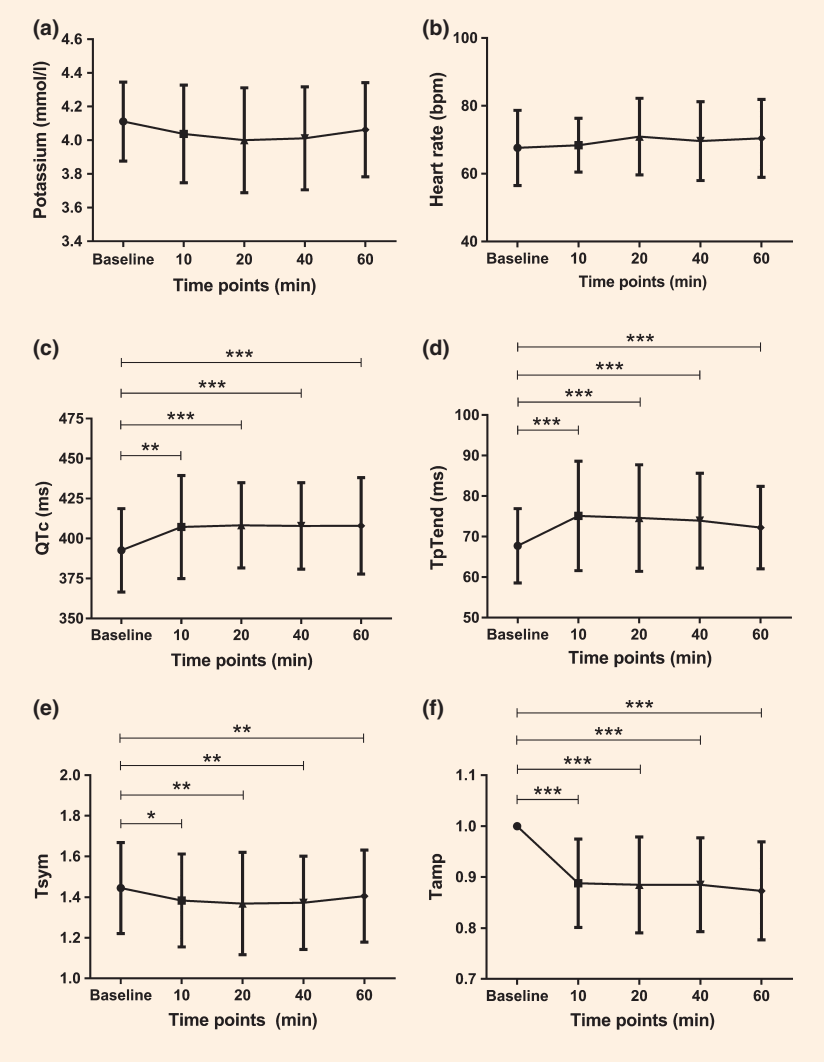


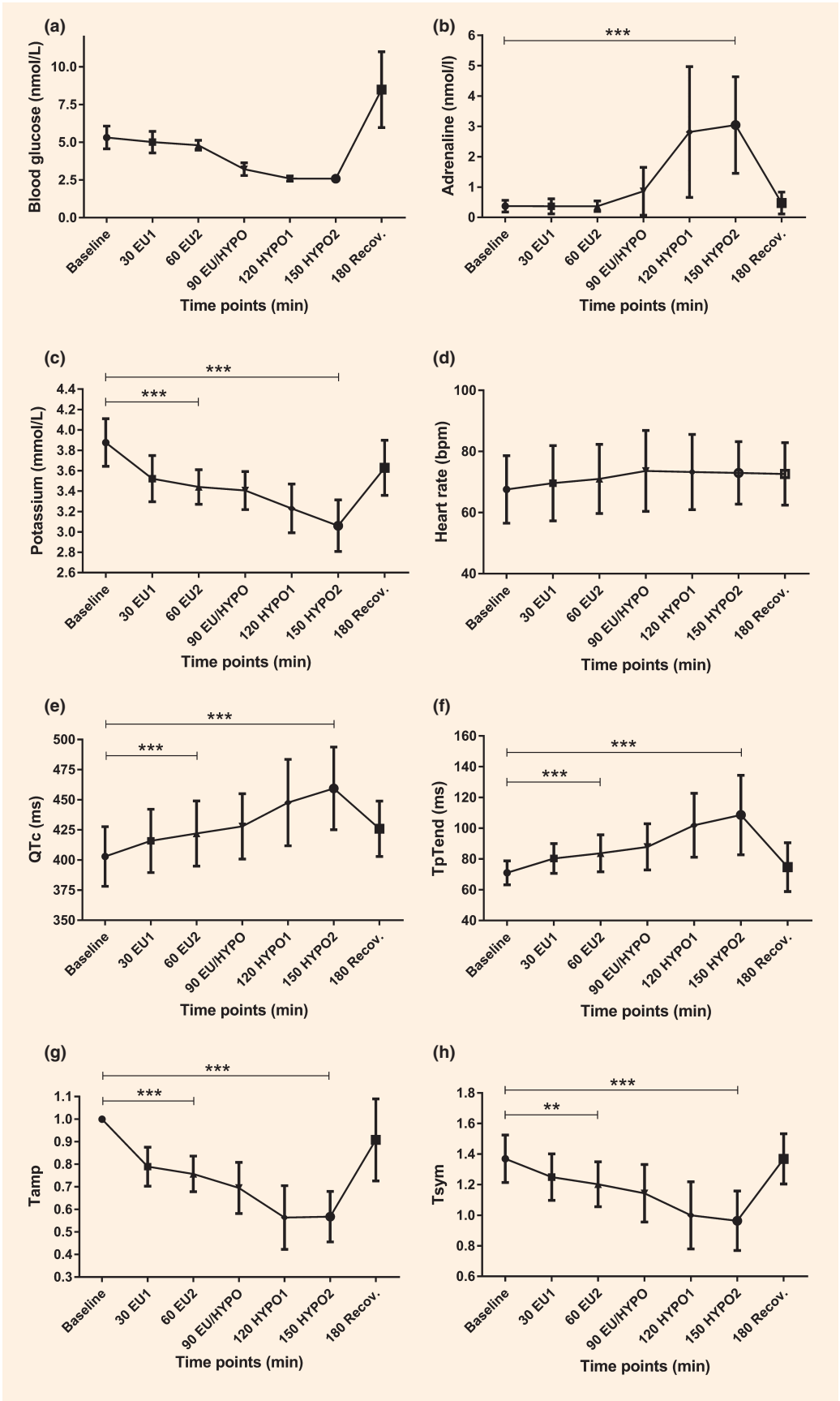
FIGURE 1 Biochemical and electrophysiological variables during salbutamol challenge. (a) Serum potassium. (b) Heart rate. (c) QT_c interval duration. (d) T_pT_{end} interval duration. (e) T-wave area symmetry (T_{sym}). (f) Normalized T-wave amplitude (T_{amp}). Repeated measures ANOVA with contrasts vs. baseline. Greenhouse–Geisser correction was used where sphericity was violated. Data are displayed as mean ± SD. *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$.

$P = 0.553$). Additionally, no statistically significant correlations were detected between the changes in T_pT_{end} ($r = 0.075$, 95% CI −0.406 to 0.524; $P = 0.767$), T_{sym} ($r = 0.355$, 95% CI −0.132 to 0.706; $P = 0.149$) or T_{amp} ($r = 0.148$, 95% CI −0.347 to 0.572; $P = 0.558$) during salbutamol challenge and hyperinsulinaemic–hypoglycaemic clamp.

Discussion

In this study we sought to determine whether nebulized β_2-adrenoreceptor agonist salbutamol induced electrophysiological changes were related in character and magnitude to hypoglycaemia-induced electrophysiological changes in young people with Type 1 diabetes.

FIGURE 2 Biochemical and electrophysiological variables during hyperinsulinaemic–hypoglycaemic clamp. (a) Serum arterialized blood glucose. (b) Serum adrenaline. (c) Serum potassium. (d) Heart rate. (e) QT_c interval duration. (f) T_pT_{end} interval duration. (g) Normalized T-wave amplitude (T_{amp}). (h) T-wave area symmetry (T_{sym}). 30 EU1 and 60 EU2, euglycaemic time points 30 and 60 min after the start of the protocol; 90 EU/HYPO, transition from euglycaemia to hypoglycaemia; 120 HYPO1 and 150 HYPO2, hypoglycaemic time points 120 and 150 min after the start of the protocol; 180 Recov, recovery time. For further details please see Materials and Methods. Repeated measures ANOVA with contrasts vs. baseline. Greenhouse–Geisser correction was used where sphericity was violated. Data are displayed as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.



We showed that administration of 2.5 mg nebulized salbutamol led to significant prolongation of QT_c interval duration with concomitant T_pT_{end} interval prolongation, flattening of the T-wave and a change towards a more symmetric T-wave. These findings are largely in keeping with previously published data indicating that salbutamol causes QT_c interval prolongation, T-wave flattening and, in some participants, ST-segment depression and emergence of U-waves [20]. To our knowledge, we are the first to report that salbutamol inhalation results in significant T_pT_{end} interval prolongation and a change in T-wave area symmetry. T_pT_{end} and T_{sym} are measures of dispersion of cardiac repolarization, an important factor in the initiation of ventricular arrhythmias [19,21] and T_pT_{end} prolongation has been shown to be associated with increased risk of sudden cardiac death [22]. In our study, we observed non-significant trends towards increased heart rate and decreased serum potassium levels. Given the previously reported significant changes in these characteristics in similar settings [23], this probably reflects the relatively small number of participants. Since the introduction of β_2-adrenoreceptor agonists in the treatment of obstructive airway disease in the 1960s, concerns have been raised regarding their short- and long-term cardiovascular safety profile and this issue has remained controversial. Case reports suggesting increased risk of cardiac arrhythmias linked with the use of salbutamol have been published [24]; however, data from larger studies and meta-analyses have been conflicting. Some studies have reported a significantly increased risk for adverse cardiovascular events [25], whereas others have not [26]. Careful use of β_2-adrenoreceptor agonists, especially in specific patient populations such as in those with underlying cardiac conditions, is therefore warranted.

During the hyperinsulinaemic euglycaemic part of the clamp we observed a decrease in plasma potassium levels with concomitant QT_c and T_pT_{end} interval prolongation and decreased T-wave amplitude (T_{amp}) and more symmetric T-waves (decreased T-wave area symmetry ratio T_{sym}). Significant increases in plasma catecholamine levels were detected during the subsequent hyperinsulinaemic–hypoglycaemic part of the clamp, which resulted in an additional decrease in plasma potassium levels, QT_c and T_pT_{end} prolongation, and decreased T_{amp} and T_{sym}. These biochemical and electrophysiological findings confirm our previous studies indicating that hypoglycaemia causes an acquired long-QT syndrome with pro-arrhythmogenic changes in T-wave morphology and that these changes are caused mainly by a combination of insulin-induced hypokalaemia and hypoglycaemia-stimulated catecholamine release [1,13]. Importantly, similar electrophysiological changes are present during both experimental [1] and clinical hypoglycaemia in various groups of individuals – in children and adolescents [27], in young and otherwise healthy people with type 1 diabetes [4] or in middle-aged people with Type 2 diabetes with cardiovascular risk factors [3]. We also detected a non-significant trend towards increased heart rate during the hypoglycaemic part of the clamp in keeping with changes observed in our previously published studies [13,14].

Lastly, we examined whether the character and magnitude of salbutamol-induced changes in cardiac repolarization correlated with changes induced by a hyperinsulinaemic–hypoglycaemic clamp. We did not find any significant correlation in any of the examined electrophysiological variables, which, at first sight and given the current knowledge about the mechanisms causing hypoglycaemia and salbutamol-induced QT_c interval prolongation, appears surprising. Clearly, one possible explanation is that with this number of participants our study lacked sufficient statistical power to demonstrate a statistically significant association. Eighteen is a relatively large number of participants for studies inducing experimental hypoglycaemia using a hyperinsulinaemic clamp and this number provided 80% power to identify a statistically significant *r*-value of 0.6. We reasoned that it would be necessary to identify this strength of association if we were to justify inhaled salbutamol as a screening test. The upper limits of the 95% CI of correlations for QT_c, T_pT_{end} and T_{amp} in our study do not include values of > 0.6, suggesting that these results are neither statistically significant nor clinically relevant. The rather wide 95% CI for T_{sym} includes the clinically relevant correlation however, which suggests that this result is not statistically significant but might be potentially clinically relevant and further studies on larger number of participants will be required.

Our hypothesis was based on our previous work which showed that QT_c interval prolongation induced by hyperinsulinaemic–hypoglycaemic clamp in healthy individuals was completely abolished by atenolol [13]. In the same individuals, keeping the serum potassium levels within the normal range (3.5–4.5 mmol/l) during a hyperinsulinaemic–hypoglycaemic clamp on a different occasion only partially diminished the magnitude of QT_c prolongation [13]. Atenolol was subsequently shown to significantly reduce QT_c prolongation during hyperinsulinaemic–hypoglycaemic clamps in individuals with Type 1 diabetes [14]. These data therefore implicated sympathoadrenal activation as a major driver of hypoglycaemia-induced QT_c prolongation. Both sympathoadrenal response, independently of its trigger, and hypoglycaemia cause hypokalaemia. Catecholamines and salbutamol cause hypokalaemia via binding to β_2-adrenoreceptors with resulting formation of cyclic adenosine monophosphate (cAMP) and subsequent stimulation of membrane-bound sodium potassium adenosine triphosphatase (Na^+/K^+-ATPase) [12]. This ion channel causes a shift of potassium from the extracellular to the intracellular compartment with resulting hypokalaemia. Hypoglycaemia causes hypokalaemia via two mechanisms: sympathoadrenal activation and via the effects of insulin, which stimulates potassium cellular uptake by elevation and increased sensitivity to intracellular sodium, translocation and activation of Na^+/K^+-ATPase and by inhibition of potassium cellular efflux [28]. Thus, mechanisms behind β_2-adrenergic and

hypoglycaemia-induced hypokalaemia are similar, but not identical. In addition, hypoglycaemia causes QT_c prolongation by direct inhibition of I_{Kr} channels in the cardiomyocyte membrane [29]. The resulting prolongation of action potential duration, together with intracellular calcium loading in the cardiomyocyte caused by increased β-adrenergic stimulation, represent the two major pro-arrhythmic pathomechanisms [5]. The one obvious difference between the salbutamol challenge and hyperinsulinaemic–hypoglycaemic clamp in our study was the presence of hypokalaemia (K^+ < 3.5 mmol/l) during the clamp. Salbutamol administration, in our hands, did not lead to hypokalaemia and potassium levels were relatively constant during the observed period of 60 min post administration. It is possible, that the effect of hyperinsulinaemia-induced sympathoadrenal activation with hypokalaemia and potential direct effect of hypoglycaemia on I_{Kr} channels [29] influenced the resulting electrophysiological changes in a given individual. Consistent with our previous observations that sympathoadrenal activation appears to be the main driver of hypoglycaemia-induced electrophysiological changes, we wonder whether the response is also modulated by hypokalaemia and direct inhibitory effect of hypoglycaemia on I_{Kr} channels.

There are other limitations to the current study. First, we could not control for the variable impairment in hypoglycaemia-induced sympathoadrenal responses among individuals with Type 1 diabetes during a given hypoglycaemic stimulus. This known heterogeneity may be an important reason for the limited correlation.

Second, we examined electrophysiological responses during experimental hypoglycaemia rather than clinical hypoglycaemia. Circulating insulin concentrations during experimental hypoglycaemia are greater than those generally observed in clinical hypoglycaemia with resulting greater electrophysiological effects due to more pronounced hypokalaemia [1,30].

Third, it is unclear to what extent variable degrees of autonomic dysfunction contributed to the lack of a relationship. Most of the participants did not have cardiovascular autonomic neuropathy (15/18), three had some degree of autonomic dysfunction as measured by cardiovascular autonomic function tests, but none had definite cardiovascular autonomic neuropathy.

In summary, we have described electrophysiological changes following a single dose of nebulized salbutamol and during a subsequent hyperinsulinaemic–hypoglycaemic clamp in 18 young people with Type 1 diabetes. Both stimuli caused prolongation of QT_c and T_pT_{end} intervals and decreased T-wave amplitude (T_{amp}) and T-wave area symmetry (T_{sym}). The magnitude of electrophysiological changes induced by these two stimuli does not correlate in a given individual, however. Salbutamol does not, therefore, appear to be a useful screening tool to assess an individual's electrophysiological response to hyperinsulinaemic hypoglycaemia. A further search for ways of identifying those with diabetes at greatest risk of QT_c lengthening and potentially hypoglycaemia-induced arrhythmias is warranted. The current emergence of continuous glucose monitoring into clinical practice and the increasing sophistication of ambulatory Holter ECG monitoring may offer a more promising approach particularly as it involves direct measurement of arrhythmias. Screening for those exhibiting arrhythmias rather than identifying a surrogate measure of abnormal cardiac repolarization such as QT interval may be a better approach in management of this rare but devastating syndrome.

Funding sources

This is a summary of independent research funded in part by the Diabetes UK grant (grant number BDA/RD:06−0003255) awarded to E.J.R. and conducted at the National Institute for Health Research (NIHR) Sheffield Clinical Research Facility. The views expressed are those of the authors and not necessarily those of Diabetes UK, NHS, the NIHR or the Department of Health.

Competing interests

S.R.H. received research grants from Medtronic UK Ltd. He has served on speaker panels for Sanofi Aventis, Eli Lilly, Takeda, NovoNordisk and Astra Zeneca for which he has received remuneration. He has served on advisory panels or as a consultant for Boeringher Ingelheim, NovoNordisk, Eli Lilly and Takeda for which his institution has received remuneration. All other authors of this work have no relevant conflict of interest to disclose.

Acknowledgements

The authors would like to thank the members of the nursing staff at the National Institute for Health Research (NIHR) Sheffield Clinical Research Facility for their help with the hypoglycaemic clamps and in particular to all study participants.

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