Normal insulin release during sustained hyperglycaemia in hypokalaemic periodic paralysis: role of the potassium channel opener pinacidil in impaired muscle strength

J. J. M. LIGTENBERG, T. W. VAN HAEFFEN*, L. E. VAN DER KOLK, A. J. SMIT†, W. J. SLUITER, W. D. REITSMA‡ and T. P. LINKS
Department of Endocrinology, University Hospital, Groningen, The Netherlands, *Department of Internal Medicine, University Hospital, Utrecht, The Netherlands, and †Department of Internal Medicine, University Hospital, Groningen, The Netherlands

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1. Hypokalaemic periodic paralysis is characterized by attacks of muscle weakness. Glucose, insulin and an abnormal regulation of ATP-sensitive potassium channels may be involved in these attacks. We studied the effect of hyperglycaemia and of the potassium channel opener pinacidil on insulin release and muscle strength in patients with hypokalaemic periodic paralysis.

2. Insulin release was assessed on two occasions in four patients with hypokalaemic periodic paralysis and in eight matched control subjects, with and without treatment with 25 mg pinacidil orally, during a hyperglycaemic glucose clamp at a blood glucose level of 10 mmol/l, in a placebo-controlled, double-blind study. Muscle strength was measured in the hypokalaemic periodic paralysis patients before and during hyperglycaemia using a handheld dynamometer.

3. During the clamp, the mean glucose concentration (10–180 min) in control subjects was 9.9 ± 0.07 and 10.0 ± 0.03 mmol/l with and without pinacidil respectively, and in patients with hypokalaemic periodic paralysis was 10.0 ± 0.04 and 10.1 ± 0.06 mmol/l respectively (not significantly different).

In both groups, the areas under the insulin curve from 0 to 10 min (first-phase insulin release) and from 30 to 180 min (second phase) were not different on the pinacidil study day compared with on the placebo day. The areas under the insulin curve of the first and second phases also did not differ between control subjects and patients with hypokalaemic periodic paralysis (with or without pinacidil). The M/I ratio, a measure of insulin sensitivity, was not different in the two groups.

On the placebo day, baseline muscle strength in patients with hypokalaemic periodic paralysis was 165 ± 16 N for the hip abductors and 168 ± 19 N for the knee flexors. During the period of hyperglycaemia on the placebo day, muscle strength did not decrease in either muscle group. On the pinacidil study day, an increase in muscle strength was found only in the two hypokalaemic periodic paralysis patients with the lowest mean muscle strength (<150 N) on the placebo day. The two hypokalaemic periodic paralysis patients with a mean muscle strength on the placebo day >150 N showed no increase in muscle strength with pinacidil.

4. Insulin secretion and sensitivity were normal in patients with hypokalaemic periodic paralysis. Hyperglycaemia during hyperglycaemic clamping did not provoke paralytic attacks and did not result in a decrease in muscle strength. The potassium channel opener pinacidil had no effect on insulin secretion in hypokalaemic periodic paralysis patients or in normal subjects. Pinacidil may enhance muscle strength in those hypokalaemic periodic paralysis patients who suffer partial paralytic attacks.

INTRODUCTION

Hypokalaemic periodic paralysis (HOPP) is a rare inherited disease characterized by transient attacks of muscle weakness of varying intensity and duration [1, 2]. During these paralytic attacks, serum potassium levels fall below normal. Although the defective gene associated with HOPP has been localized on chromosome 1q31–32, the region to which the α-subunit of the dihydropyridine receptor calcium channel also maps [3], it is not clear how disturbances of this channel can result in the clinical picture of paralysis. Carbohydrate-rich meals, rest after exercise, and cold and stress are known provocative factors, but it is not clear why provocation with carbohydrates or glucose results in paralytic attacks in these patients. Several authors...
[4–6] have suggested impairment of glucose tolerance as a mechanism of HOPP, and the authors of one study proposed that glucose infusion causes strength impairment in HOPP patients [7]. On the other hand, in a hyperinsulinaemic euglycaemic clamp study, hyperinsulinaemia was thought to be the most important factor in provoking paralysis [8]. And in another study, increased first-phase insulin secretion during an intravenous glucose tolerance test (GTT) was found in HOPP patients, suggesting an abnormal regulation of ATP-sensitive potassium channels (K⁺[ATP] channels) in the pancreatic β-cell [9]. In that study, it was also found that the antihypertensive drug pinacidil, an ATP-sensitive potassium channel opener [10, 11], prevented muscle weakness after glucose loading. In in vitro experiments with muscle fibres from HOPP patients, the potassium channel opener cromakalim has been found to increase contraction force [12]. Thus, there is support for a role of K⁺[ATP] channels in both the muscular abnormalities and the possible hyperinsulinaemia in HOPP patients. As K⁺[ATP] channels are present in the sarcolemma of skeletal muscle cells [10, 13], opening of these channels with pinacidil might result in an enhancement of muscle strength in HOPP patients. K⁺[ATP] channels are also present in the pancreatic β-cell; closure of these channels leads to accumulation of intracellular K⁺ and an increase in insulin secretion, whereas opening of these channels decreases insulin release [14, 15]. Pinacidil inhibits insulin release in vitro in pancreatic β-cells [14, 16, 17] and has been reported to diminish first-phase glucose-induced insulin release in healthy subjects [18].

It is necessary to determine firstly if an important role in the pathophysiology of HOPP can be ascribed to impaired insulin secretion caused by abnormal behaviour of β-cell K⁺[ATP] channels and, secondly, if such abnormalities in K⁺[ATP] channels may also be present in muscle cells. We therefore undertook a placebo-controlled, double-blind investigation in four HOPP patients, using the hyperglycaemic glucose clamp technique, to assess the effects of pinacidil on hyperglycaemia-induced insulin release. At the same time, we investigated the effect of pinacidil on muscle strength during hyperglycaemia, which has been suggested to cause muscle weakness or even paralysis. For comparison of hyperglycaemia-induced insulin release, we used data from eight age-, sex- and body mass index (BMI)-matched control subjects studied previously.

**METHODS**

**Subjects (Table 1)**

The study was approved by the local medical ethics committee and all participants gave written informed consent. Four HOPP patients from one family covering five generations [2] were recruited. For each HOPP patient, two age-, sex- and BMI-matched healthy subjects were sought. These control subjects had taken part in a larger study on the influence of pinacidil on glucose-induced insulin release. Exclusion criteria were kidney or liver dysfunction, pregnancy, a family history of diabetes mellitus, use of any medication; except for the HOPP patients, use of potassium chloride in a slow-release formula. Other medication (such as acetazolamide) was stopped at least 3 weeks before this study. Mean age, BMI and sex distribution were similar in both groups (Table 1). All subjects studied had a normal fasting blood glucose (<5.6 mmol/l).

**Protocol**

The participants were instructed to maintain their usual diet and to refrain from alcohol consumption on the day before the study. They were studied on two occasions separated by at least 7 days with a randomized, double-blind study design. The subjects had fasted since 22.00 h on the previous day. From 08.00 h onwards they remained in the supine position in a quiet environment. An intravenous cannula was inserted in the antecubital vein of each arm. One line was used for blood sampling and the other for infusion of 20% dextrose. Pinacidil (25 mg of a long-acting micropelleted formulation; Leo Pharmaceutical Products, Copenhagen, Denmark) or placebo was taken 1 h before the start of the clamp. After 1 h rest, baseline blood samples were taken. Subsequently, a hyperglycaemic glucose clamp was performed over 180 min, starting with an intravenous bolus injection of 20% dextrose (30 mg/kg over 90 s for each 1 mmol/l intended increase in blood glucose level [19]) and followed by a variable infusion of 20% dextrose [20] with the aim of maintaining blood glucose at 10 mmol/l. To each 500 ml of dextrose was added 10 mmol of potassium chloride to ensure that plasma K⁺ would not drop dramatically because it is well known that insulin stimulates K⁺ uptake. Mixed venous blood glucose was measured at −30, −15, 0, 2, 5, 7, 10, 12 and 15 min and at 5-min intervals thereafter. Blood

| Table 1. Baseline subject characteristics (means ± SEM) of four HOPP patients and eight matched control subjects. EMG, electromyography; MFCV, muscle fibre conduction velocity. |
|-------------|-------------------|-------------------|
| Age (years) | 47 ± 3.7          | 47 ± 1.6          |
| Gender (M/F)| 1/3               | 2/6               |
| Body mass index (kg/m²) | 23.8 ± 1.2 | 23.8 ± 0.7 |
| Fasting blood glucose (mmol/l) | 4.7 ± 0.2 | 4.9 ± 0.2 |
| Fasting plasma insulin (pmol/l) | 64.2 ± 19.7 | 51.1 ± 10.9 |
| Fasting plasma C-peptide (pmol/l) | 0.56 ± 0.09 | 0.48 ± 0.08 |
| Serum potassium (mmol/l) | 4.6 ± 0.2 | 4.3 ± 0.1 |
| Systolic blood pressure (mmHg) | 124 ± 6 | 127 ± 5 |
| Diastolic blood pressure (mmHg) | 71 ± 4 | 79 ± 3 |
| Surface EMG (MFCV, m/s, n < 3.8) | 3.2 ± 0.15 | Not done |
samples for plasma insulin and C-peptide were taken at -30, -15, 0, 2, 5, 7, 10, 20, 30, 60, 90, 120, 150 and 180 min. Blood samples for measurement of serum potassium were drawn at baseline and after 60, 120 and 180 min.

In the HOPP patients, the muscle strength of the hip abductors and the knee flexors (both left and right) was measured at baseline and at hourly intervals by the same investigator with a hand-held dynamometer in a standard position; the repeatability of this method is described as good, with a mean week-to-week difference of less than 20% and a ratio of variation between two measurements within one study day of 0.85–1.22, with a coefficient of variation of 8.9% [21].

Analytical procedures

Plasma insulin and C-peptide were assayed by radioimmunoassays (Novo Nordisk Immunoch- emical Department, Copenhagen, Denmark). Blood glucose was measured with a Yellow Springs glucose analyser (model 23A, Yellow Springs, Yellow Springs, OH, U.S.A.). Serum potassium was measured routinely using a Technicon SMAC analyser.

Mean and SD blood glucose levels between 10 and 180 min of each individual clamp were calculated. The SD was divided by the mean blood glucose from 10 to 180 min ( = coefficient of variation) to estimate the precision of the clamp. The area under the plasma insulin curve (AUC_insulin) from 0 to 10 min and from 30 to 180 min was calculated with the trapezoidal rule as a measure of the first and second phases of insulin secretion during the clamp.

The mean glucose infusion rate (M) (μmol min⁻¹ kg⁻¹) during the second and third hours of the clamp was used as an index of glucose metabolism [20]. The mean plasma insulin levels at 60–120 min and 120–180 min during steady-state hyperglycaemia (I) (pmol/l) were used to calculate the M/I ratio, which is the amount of glucose metabolized per minute per unit of plasma insulin concentration (μmol min⁻¹ kg⁻¹ per pmol/l). The M/I ratio calculated in this manner during a hyperglycaemic clamp provides an estimate of insulin sensitivity, and is strongly correlated with the M/I ratio measured during a euglycaemic hyperinsulinaemic clamp, the so-called gold standard for insulin sensitivity [22].

Results are expressed as means±SEM unless stated otherwise. Between-group comparisons were made using the unpaired Wilcoxon test. Within-group changes in parameters were evaluated using the paired Wilcoxon test on Friedman’s two-way analysis of variance (ANOVA) where appropriate. A two-sided P-value less than 0.05 was taken as significant.

RESULTS

Glucose concentrations

Fasting blood glucose levels were not different in the two groups (Table 1). Five minutes after the start of the hyperglycaemic clamp, blood glucose levels with and without pinacidil rose to 9.2±0.39 and 9.5±0.31 mmol/l respectively in control subjects and 9.0±0.56 and 9.1±0.22 mmol/l respectively in HOPP patients (not significantly different). During the period of sustained hyperglycaemia (10–180 min) the mean averaged glucose concentration with and without pinacidil was 9.9±0.07 and 10.0±0.03 mmol/l respectively in the control subjects and 10.0±0.04 and 10.1±0.06 mmol/l respectively in the HOPP patients (not significantly different within and between groups). The coefficient of variation, reflecting the stability of the blood glucose concentration during the clamp, with and without pinacidil, averaged 5.1±0.4% and 5.0±0.8% respectively in the control group and 3.7±0.7% and 4.4±0.8% respectively in the HOPP group (not significantly different).

Insulin release (Fig. 1)

Fasting plasma insulin and C-peptide levels were not significantly different between HOPP patients and controls (Table 1). Plasma insulin responses were biphasic, with a peak at 5 min and a gradual increase until 180 min, the end of the study period. Plasma insulin levels after 5 min, with and without pinacidil, rose to 258±55.4 and 273±71.1 pmol/l respectively (not significantly different) in the control group and to 232±41.2 and 221±43.6 pmol/l respectively (not significantly different) in the HOPP patients. Plasma C-peptide levels after 5 min, with and without pinacidil, rose to 1.47±0.36 and 1.58±0.29 nmol/l respectively in the control group and to 1.25±0.09 and 1.21±0.16 nmol/l respectively in the HOPP patients (not significantly within and between groups).

In both groups, AUC_insulin from 0 to 10 min (first phase) and from 30 to 180 min (second phase) were not different on the pinacidil study day compared with the placebo day. There was also no difference in AUC_insulin of the first and second phases between controls and HOPP patients, with or without pinacidil.

The M/I ratio, a measure of insulin sensitivity, during the second hour of the clamp, with and without pinacidil, was 0.27±0.03 and 0.24±0.02 μmol kg⁻¹ min⁻¹ per pmol/l respectively in control subjects and 0.20±0.04 and 0.17±0.03 μ mol kg⁻¹ min⁻¹ per pmol/l respectively in HOPP patients (not significantly different within and between groups). The M/I ratio during the third hour of the clamp, with and without pinacidil, was 0.31±0.04 and 0.23±0.02 μmol kg⁻¹ min⁻¹ per pmol/l respectively in control subjects and
0.22 ± 0.05 and 0.24 ± 0.05 pmol kg⁻¹ min⁻¹ per pmol/l respectively in HOPP patients (not significantly different within and between groups). Thus, in both groups, insulin sensitivity was not significantly different on the placebo day and did not change significantly under the influence of pinacidil.

**Serum potassium**

Serum potassium levels remained stable on both days, being at baseline, with and without pinacidil, 4.2 ± 0.16 and 4.3 ± 0.10 mmol/l respectively in the control group and 4.2 ± 0.04 and 4.6 ± 0.18 mmol/l respectively in HOPP patients. After 180 min the corresponding values were 4.1 ± 0.09 and 4.2 ± 0.10 in the control group and 4.3 ± 0.11 and 4.6 ± 0.31 mmol/l in HOPP patients (not significantly different).

**Muscle strength in HOPP patients (Table 2)**

On the placebo day, muscle strength (mean of right and left leg measurements) before the start of intravenous glucose loading was 165 ± 16 N for the hip abductors and 168 ± 19 N for the knee flexors. During the hyperglycaemic glucose clamp, none of the patients complained about muscle paresis, muscle pain or cramps. During the period of sustained hyperglycaemia (10–180 min) the muscle strength did not change in either the hip abductors or the knee flexors. On the pinacidil study day, an increase in Δ muscle strength was found compared with the muscle strength on the placebo day; the change in muscle strength on the pinacidil study day showed a significant negative correlation (r = -0.95, P < 0.01) with muscle strength on the placebo study day (Table 2). In other words, muscle strength was enhanced by pinacidil in the two HOPP patients with the lowest muscle strength on the placebo day (<150 N), whereas muscle strength did not change in the two HOPP patients with a better 'baseline' muscle strength. Overall, on the pinacidil study day, the muscle strength of the hip abductors was 160 ± 14 N at baseline and 183 ± 5 N after 3 h (not significant), and that of the knee flexors was 186 ± 12 N at baseline and 201 ± 6 N after 3 h (not significant). During the days after the studies, the patients had no complaints of paralysis.

**DISCUSSION**

The pathophysiological mechanism of HOPP is not yet clear: one hypothesis is that the ratio of the conductances of Na⁺ and K⁺ across the skeletal muscle cell membrane is abnormal [1]. Another hypothesis is based on the provocative action of carbohydrate meals, suggesting a role for insulin secretion. ATP-sensitive potassium channels may play a connecting role in linking insulin secretion to disturbances in cell membrane function. Glucose, the main regulator of insulin release, is — after entering the pancreatic β-cell — rapidly degraded, causing an increase in intracellular [ATP], closure of K⁺[ATP] channels and depolarization of the cell membrane. This allows Ca²⁺ to enter the cell through voltage-dependent calcium channels, resulting in insulin secretion. It has been suggested that disturbances in K⁺[ATP] channels in pancreatic β-cells may be involved in abnormal insulin secretion [9], and disturbances in K⁺[ATP] channels in the striated muscle cells could be involved in the effects of potassium channel openers on muscle strength in vitro and in vivo [9, 12, 23]. It has also been suggested that the K⁺[ATP] channels of pancreatic β-cells contain a sulphonylurea-binding domain, a binding site for potassium channel openers and binding sites that can discriminate between ATP and ADP. More recently, cloning of this K⁺[ATP] channel has revealed that it is a complex of the sulphonylurea receptor and a potassium channel [24]. It is not known if this complex in this form is present in K⁺[ATP] channels in striated muscle cells. However, as potassium channel openers may have a membrane-stabilizing effect in muscle diseases as well as an effect on insulin secre-
Insulin release and hypokalaemic periodic paralysis

Table 2. Muscle strength of knee flexors and hip abductors (at baseline and at 180 min) in four HOPP patients during a 3 h hyperglycaemic glucose clamp. Data are given in Newtons and are means of left and right leg measurements.

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tion, we investigated the effect of pinacidil on muscle strength and insulin secretion.

In the present study, the hyperglycaemic glucose clamp technique was used for assessment of insulin release in HOPP and control subjects, because it yields more precise data than intravenous GTTs [20, 25, 26], previously used in our studies. We did not use the ‘hot box’ method to arterialize venous blood, so the mixed venous blood glucose levels that we measured underestimate arterialized blood glucose levels by approximately 0.5 mmol/l [27]. Fasting blood glucose concentrations were the same in HOPP patients and control subjects. Mean glucose levels achieved during the clamps were not different between the two groups. Both first- and second-phase insulin release turned out to be the same in HOPP patients and in control subjects. Thus, the previous suggestion, based on results of intravenous GTT studies, that first-phase insulin secretion is increased in HOPP patients [9] could not be confirmed. Although in a previous study with pinacidil we found that glucose tolerance deteriorated at lower plasma insulin levels during an intravenous GTT in healthy subjects [28], no effect of pinacidil on insulin secretion was found in the present study. As the intravenous GTT studies gave conflicting results, and as a hyperglycaemic clamp study yields more precise results because of the stability of blood glucose levels during the whole clamp, we believe that the results of the present study are more reliable.

Insulin sensitivity, expressed as the $M/I$ ratio, was the same in the two groups. This means that the reaction of the pancreatic $\beta$-cell to a glucose stimulus and the subsequent insulin-mediated uptake of glucose in various cells appears not to be grossly abnormal in HOPP patients. Owing to the relatively small number of HOPP patients studied, firm conclusions about the effect of pinacidil on the $M/I$ ratio cannot be drawn. In patients with essential hypertension, we found an indication that pinacidil might enhance insulin sensitivity, which could be due to the vasodilatory effect of this drug [29].

In addition, we studied the effect of hyperglycaemia/hyperinsulinaemia, induced by hyperglycaemic glucose clamping, on muscle strength in HOPP patients. During 3 h hyperglycaemia on the placebo study day, no deterioration in muscle strength was observed: average baseline and 3-h muscle strength were the same. Moreover, none of the individual HOPP patients showed an acute decrease in muscle strength during hyperglycaemia.

In these studies, the potassium channel-opening drug pinacidil (25 mg of a long-acting micropelleted formulation; pharmacokinetics show a peak plasma concentration 1 h after ingestion and maintenance of this concentration for 4–6 h [30]) was administered acutely, 1 h before the clamp, in a randomized placebo-controlled manner. The muscle strength on the pinacidil study day in the two HOPP patients with the lowest muscle strength (<150 N) increased compared with the mean measurements
on the placebo day. In particular, the muscle strength of the knee flexors, which are known to be the most vulnerable muscle group [2, 31], was increased. The fact that baseline knee flexor strength was higher on the pinacidil study day than on the placebo study day could be because pinacidil was administered 1 h before the first muscle strength measurements, and is supportive of pinacidil's effect. Because the study medication was randomized, a training or learning effect on muscle strength is not likely. These findings would suggest an effect on muscle membrane stability, by opening of K⁺[ATP] channels in skeletal muscle cells by pinacidil, with a subsequent increase in contraction force. This is in agreement with experiments in vitro, in which the K⁺ channel openers cromakalim and pinacidil increase K⁺ transport over the cell membrane in skeletal muscle biopsies [12, 32]. The K⁺[ATP] channel blocker tolbutamide inhibits this effect, strongly suggesting that it is mediated by K⁺[ATP] channels [32]. In experiments in vitro with muscle fibres from HOPP patients, the potassium channel opener cromakalim increases contraction force [12]. Because membrane potential stabilization and normalization of the abnormalities in the mechanical properties of skeletal muscle have also been found during treatment with potassium channel-opening drugs in other muscle diseases [33], a common effect on muscle membrane stability could be present, independently of disease-specific membrane abnormalities.

The defective gene associated with HOPP is located on chromosome 1 and codes for a calcium channel that inactivates the so-called L-type calcium channel [3]. How this inactivation can lead to paralytic attacks in HOPP can only be speculated upon. The membrane depolarization that occurs in HOPP [34] may result from disturbances in this Ca²⁺ channel, and may be compensated by an alteration in the state of the K⁺[ATP] channels, possibly resulting from altered feedback of Ca²⁺ in K⁺[ATP] channels [15].

In conclusion, insulin secretion (and sensitivity) were normal in four HOPP patients. Hyperglycaemia did not provoke paralytic attacks and did not result in a decrease in muscle strength in HOPP patients. The potassium channel opener pinacidil had no effect on insulin secretion in HOPP patients or in normal subjects. Our findings suggest that pinacidil may enhance muscle strength in HOPP patients, but long-term intervention studies with potassium openers are needed to evaluate the effect on muscle strength and on the frequency of paralytic attacks.

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