Abnormal endothelin B receptor vasomotor responses in patients with Hirschsprung’s disease

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Received 2 November 2001 and in revised form 6 December 2001

Summary

Background: Hirschsprung’s disease is associated with defects in the endothelin-3 and endothelin B receptor genes.

Aim: To assess the in vivo vasomotor responses to endothelin B receptor stimulation in patients with Hirschsprung’s disease.

Methods: Forearm blood flow was measured using venous occlusion plethysmography in 10 patients with Hirschsprung’s disease and 10 matched healthy controls during intra-brachial infusion of the highly selective endothelin B receptor agonist, sarafotoxin S6c. To simulate endothelin B receptor dysfunction, sarafotoxin S6c was co-infused with the highly selective endothelin B receptor antagonist, BQ-788, in six of the healthy controls.

Results: Sarafotoxin S6c caused a brief initial vasodilatation followed by a slow-onset, sustained vasoconstriction (p<0.001). Compared to control subjects, patients with Hirschsprung’s disease had a substantial impairment of the initial vasodilatation whilst producing a more pronounced subsequent vasoconstriction (p<0.001). In healthy controls, co-infusion of BQ-788 and sarafotoxin S6c caused a similar pattern of responses to those obtained in patients with Hirschsprung’s disease: abolition of the initial vasodilatation and augmentation of subsequent vasoconstriction (p<0.001).

Discussion: In the majority of patients with Hirschsprung’s disease, there is a functional defect of the vascular endothelin B receptor.

Introduction

Hirschsprung’s disease is a polygenic inherited condition that is characterized by the absence of ganglionic neuronal tissue in the distal colon and usually presents in childhood with chronic severe constipation. It has an incidence of 1 in 5000 live births and generally requires surgical excision of the affected bowel segment. Despite the heterogenous genetic basis for this disorder, several defects have so far been linked to Hirschsprung’s disease: the c-ret proto-oncogene, the endothelin B (ETB) receptor gene and the endothelin-3 gene. Animal gene targeting studies suggest that endothelin-3 and the ETB receptor may play a significant role. Several groups have reported endothelin-3 and ETB receptor mutations in both familial and isolated cases of Hirschsprung’s disease. In addition to these mutations, the developmental endothelin-3/ETB receptor interaction may be impaired at any number of levels, such as a deficiency in endothelin-3 or an impairment of the second messenger pathway of the ETB receptor, possibly also involving the c-ret proto-oncogene. However, due to a lack of appropriate methodology, there has been no functional assessment of ETB receptor function in vivo in patients with Hirschsprung’s disease.

The endothelins are a family of extremely potent vasoconstrictor peptides, with endothelin-1 contributing to the maintenance of basal vascular tone.
and blood pressure in man.\textsuperscript{13} The development of new tools to assess the role of endothelin A (ET\textsubscript{A}) and ET\textsubscript{B} receptor subtypes has provided a better understanding of their \textit{in vivo} function. Sarafotoxin S6c and BQ-788, highly selective peptidic ET\textsubscript{B} receptor agonist and antagonist, respectively, have recently become available for clinical use and provide a potential method to assess the functional activity of the ET\textsubscript{B} receptor \textit{in vivo} in man.

The aims of the present study were therefore: first, to describe the peripheral vascular response to intra-arterial sarafotoxin S6c infusion in patients with Hirschsprung’s disease and healthy matched control subjects; and second, to examine the response to sarafotoxin S6c infusion during simulated ET\textsubscript{B} receptor dysfunction using co-infusion of BQ-788 in the healthy controls.

**Methods**

**Patients and controls**

Ten adult patients with Hirschsprung’s disease were recruited through the National Health Service in Scotland database and liaison with the Paediatric Surgical Department at the Royal Hospital for Sick Children in Edinburgh, and were compared to 10 individually age- and sex-matched healthy controls recruited from our volunteer database. The study was undertaken with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki. The written informed consent of each subject was obtained before entry into the study.

All subjects were normotensive without a history of diabetes mellitus or vascular disease. Female subjects were studied between day 9 and 12 of the menstrual cycle. None of the subjects received vasoactive or non-steroidal anti-inflammatory drugs in the week before the study, and all abstained from alcohol for 24 h before the study, and from food, tobacco and caffeine-containing drinks on the day of the study. All studies were performed in a quiet, temperature-controlled room maintained at 22–25°C.

**Intra-arterial drug administration**

The brachial artery of the non-dominant arm was cannulated with a 27-standard-wire-gauge steel needle (Cooper’s Needle Works) under local anaesthesia. The cannula was attached to a 16-gauge epidural catheter (Portex) and patency maintained by infusion of saline (0.9\%: Baxter Healthcare) via an IVAC P1000 syringe pump (IVAC). The total rate of intra-arterial infusions was maintained constant throughout all studies at 1 ml/min. Pharmaceutical-grade sarafotoxin S6c (Clinalfa) and BQ-788 (Clinalfa) were administered following dissolution in saline.

**Measurements**

Blood flow was measured in both forearms by venous occlusion plethysmography as previously described.\textsuperscript{14,15} Blood pressure was monitored in the non-infused arm at intervals throughout each study using a semi-automated non-invasive oscillometric sphygmomanometer (Takeda UA 751).

**Study design**

On each occasion, subjects attended fasted and rested recumbent throughout each study. Strain gauges and cuffs were applied, and the brachial artery of the non-dominant arm was cannulated. Forearm blood flow was measured every 10 min. Saline was infused for the first 30 min to allow time for equilibration. The final blood flow measurement during saline infusion was taken as the basal forearm blood flow.

Ten patients with Hirschsprung’s disease and 10 healthy control subjects received an intra-brachial infusion of sarafotoxin S6c at 60 pmol/min for 5 min. Forearm blood flow was measured continuously for 1 h prior to, during, and for 30 min after sarafotoxin S6c infusion.

Six of the control subjects attended on each of two separate occasions and received an intra-brachial infusion of saline placebo or BQ-788 for 1 h prior to, during, and for 30 min after sarafotoxin S6c infusion at 60 pmol/min for 5 min. The order of saline placebo and BQ-788 was randomized in a double-blind manner.

**Data analysis and statistics**

Plethysmographic data were extracted from the Chart data files and forearm blood flows were calculated for individual venous occlusion cuff inflations by use of a template spreadsheet (Excel v5.0; Microsoft). The percentage change in forearm blood flow following drug administration was calculated as follows:

\[
100\% \times \frac{F(i)_{d}/F(ni)_{d} - F(i)_{v}/F(ni)_{v}}{F(i)_{v}/F(ni)_{v}}
\]

where \(F(i)\) and \(F(ni)\) represent measured blood flows in the infused and non-infused arms respectively during periods of drug (\(d\)) and vehicle (\(v\)) administration.\textsuperscript{14}

Data were examined by ANOVA with repeated measures and Wilcoxon signed rank test with
StatView v5.0.1 (SAS). Where ANOVA demonstrated significant differences in responses, post hoc comparisons were made using paired t-test (StatView v5.0.1). Unless otherwise stated, all results are expressed as mean ± SEM. Statistical significance was taken at the 5% level.

**Results**

There were no significant changes in blood pressure or heart rate, or blood flow in the non-infused forearm, during the course of the studies. Infusions were well tolerated without any adverse effects. There were no significant baseline differences between the patients and control subjects (Table 1).

**Sarafotoxin S6c and BQ-788**

Bolus administration of sarafotoxin S6c caused a bimodal vasomotor response with a brief initial vasodilatation followed by a slow onset and sustained vasoconstriction (Figure 1, ANOVA, \( p < 0.001 \) for time course of change in flow blood).

In patients with Hirschsprung’s disease, there was a substantial impairment of the initial vasodilatation response to sarafotoxin S6c followed by a more pronounced subsequent vasoconstriction (\( p < 0.001 \); ANOVA, patients vs. controls). Seven of the ten patients were below the lower 95% CI for vasodilatation to sarafotoxin S6c in the healthy volunteers.

In the six healthy control subjects, co-infusion of BQ-788 abolished the initial vasodilatation (\( p < 0.001 \); ANOVA, saline vs. BQ-788) and augmented the subsequent sustained vasoconstriction to sarafotoxin S6c (Figure 2).

**Table 1** Baseline subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29 ± 3</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>Sex (male : female)</td>
<td>7 : 3</td>
<td>7 : 3</td>
</tr>
<tr>
<td>Heart rate (/min)</td>
<td>66 ± 3</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 ± 6</td>
<td>119 ± 4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70 ± 3</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>Forearm blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-infused arm (ml/100 ml/min)</td>
<td>3.6 ± 0.3</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>Infused arm (ml/100 ml/min)</td>
<td>4.4 ± 0.7</td>
<td>3.9 ± 1.0</td>
</tr>
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Data are means ± SEM.

**Figure 1.** Percentage change in forearm blood flow following intra-brachial administration of sarafotoxin S6c (300 pmol over 5 min). Means ± SEM. a Six healthy male volunteers with (■) and without (□) co-infusion of the selective ET\(_B\) receptor antagonist, BQ-788 (1 nmol/min). b Ten patients with Hirschsprung’s disease (●) and ten age- and sex-matched controls (○). \( p < 0.001 \) (ANOVA) for the time course of the blood flow response; \( p < 0.001 \) (ANOVA) for the between-group comparison. *\( p < 0.05 \), †\( 0.10 > p > 0.05 \); paired \( t \)-test for between-group comparison at each time point.
Discussion

We report, for the first time, the in vivo assessment of ET_β_ receptor function in patients with Hirschsprung’s disease using forearm venous occlusion plethysmography and intra-arterial infusion of the highly selective ET_β_ receptor agonist, sarafotoxin S6c. In the majority of patients with Hirschsprung’s disease, there is an abnormal response to sarafotoxin S6c infusion that displays a marked similarity to the response seen during co-infusion of the ET_β_ receptor antagonist, BQ-788, in healthy controls. This suggests that there is a defect in the ET_β_ receptor pathway in the majority of patients with Hirschsprung’s disease.

The vascular ET_β_ receptor is predominantly expressed on the endothelial cell surface although some expression has also been detected on vascular smooth muscle cells. The initial increase in forearm blood flow caused by sarafotoxin S6c infusion is the consequence of ET_β_-receptor-mediated endothelium-dependent vasodilatation from nitric oxide release. This vasodilatation was abolished by co-infusion of the ET_β_ receptor antagonist, BQ-788, and was markedly attenuated in the majority of the patients with Hirschsprung’s disease. This indicates that in patients with Hirschsprung’s disease, the abnormal response to sarafotoxin S6c infusion is likely to be the result of endothelial ET_β_ receptor dysfunction.

The subsequent slow onset and sustained vasoconstriction seen with sarafotoxin S6c infusion is likely to represent either stimulation of the vascular smooth muscle ET_β_ receptor or displacement of endogenous endothelin-1 from the ET_β_ receptor, resulting in ET_α_-receptor-mediated vasoconstriction. This latter explanation is supported by the finding that plasma endothelin-1 concentrations rise following systemic ET_β_ receptor antagonism.

The ET_β_ receptor, therefore, appears to function as a clearance receptor, and since endothelin-1 contributes to basal vascular tone, displacement of endothelin-1 from the ET_β_ receptor will have vasoconstrictor effects.

The vasoconstriction in response to sarafotoxin S6c was enhanced in the patients with Hirschsprung’s disease. This is likely to reflect the removal of a tonic ET_β_-receptor-mediated endothelial vasodilatation, thereby augmenting the ET_α_-receptor-mediated vasoconstriction from

Figure 2. Percentage change in forearm blood flow at 2, 7, 20 and 30 min following intra-brachial administration of sarafotoxin S6c (300 pmol over 5 min). Range, interquartile range (box) and median (central marker). a Six healthy male volunteers with (open boxes) and without (grey boxes) co-infusion of the selective ET_β_ receptor antagonist, BQ-788 (1 nmol/min). b Ten patients with Hirschsprung’s disease (open boxes) and ten age- and sex-matched controls (grey boxes). *p<0.05, †0.10>p>0.05; Wilcoxon signed rank test.
displacement of endothelin-1 from the clearance receptors. This is consistent with the similar findings in healthy controls during co-infusion of BQ-788.

In conclusion, we report, for the first time, a functional ETB receptor defect that is present in the majority of patients with Hirschsprung’s disease. Although Hirschsprung’s disease is a polygenic condition, there may be a common pathway that is responsible for the phenotype involving a defect in the endothelin 3/ETB receptor interaction and down stream signal transduction pathways.

References