Moderate hypothermia induces a preferential increase in pancreatic islet blood flow in anesthetized rats

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Hultström M, Jansson L, Bodin B, Källskog Ö. Moderate hypothermia induces a preferential increase in pancreatic islet blood flow in anesthetized rats. Am J Physiol Regul Integr Comp Physiol 293: R1438–R1443, 2007.—The aim of the study was to characterize the effects of induced moderate hypothermia on splanchnic blood flow, with particular reference to that of the pancreas and the islets of Langerhans. We also investigated how interference with the autonomic nervous system at different levels influenced the blood perfusion during hypothermia. For this purpose, hypothermia (body temperature of 28°C) was induced by external cooling, whereas normothermic (37.5°C) anesthetized Sprague-Dawley rats were used as controls. Some rats were pretreated with either propranolol, yohimbine, atropine, hexamethonium, or a bilateral abdominal vagotomy. Our findings suggest that moderate hypothermia elicits complex, organ-specific circulatory changes, with increased perfusion noted in the pylorus, as well as the whole pancreas and the pancreatic islets. The pancreatic islets maintain their high blood flow perfusion through mechanisms involving both sympathetic and parasympathetic mediators, whereas the increased pyloric blood flow is mediated through parasympathetic mechanisms. Renal blood flow was decreased, and this can be prevented by ganglionic blockade and is also influenced by β-adrenoceptors.

pancreatic blood flow; renal blood flow; intestinal blood flow; adrenal blood flow

MILD TO MODERATE HYPOTHERMIA is used in clinical medicine to benefit from the imposed decrease in tissue metabolism and oxygen consumption (16, 28, 44). Thus hypothermia will allow for more extended time periods of surgery without hypoxic damage to tissues (43, 45). Another condition when induced hypothermia might be of relevance is during harvesting of organs for transplantation from brain dead donors, i.e., patients with abnormal body temperature regulation (53). In addition to this, cold preservation solutions are flushed through the organs intended for implantation, leading to profound changes in flow distribution (47).

Hypothermia per se is associated with changes in blood flow in several organs; this change is most commonly a decrease. This can be due to the organ’s decreased metabolism, by affecting neural control and transmission (16, 28, 29, 44), or due to changes in blood viscosity and hematocrit (27). The response of the pancreatic islet vasculature is different from that of most other organs on stimulation or inhibition of adrenoceptors. Thus activation of β2-adrenoceptors stimulates insulin release but decreases islet blood flow (22, 25), whereas inhibition of α2-adrenoceptors or all β-adrenoceptors increases islet blood flow (26). However, islet blood flow has not been studied during hypothermia.

Furthermore, moderate hypothermia consistently reduces renal blood flow (9, 10). The effects on splanchnic blood flow are more controversial, and both increased and decreased blood perfusion have been reported (13, 27). In previous studies in anesthetized rats, moderate hypothermia, i.e., body temperatures within the range of 25–30°C (16), led to an unchanged (27), a reduced (19, 38), or increased blood flow (39). In combination with hypothermic cardiopulmonary bypass procedures, there were no changes or decreases in intestinal mucosal blood flow and total pancreatic blood flow (31, 34, 37, 40, 42). In view of these divergent results, it is likely that several factors, including choice of experimental animal or anesthesia, influence the measurements.

It has long been known that cooling induces a sympathetically mediated generalized vasoconstriction in skin, fat, and skeletal muscle (18, 46). It can be speculated that this may cause a redistribution of blood favoring the splanchnic vascular beds. It should be noted in this context that many steps of catecholamine synthesis and release (5, 41, 50) are affected by hypothermia. Furthermore, cooling may in itself alter the affinity of different drugs for their receptors (7, 54). It has been reported that there is an increased potency of norepinephrine as a constrictor at a body temperature of 28°C compared with normothermia (2). Furthermore, studies on adrenoceptors during hypothermia have demonstrated a hypersensitivity in β1-receptors, but not β2, (54), and that α1-adrenoceptor-mediated vasoconstriction is attenuated, whereas that in response to α2-adrenoceptors is unaffected (12). It should be noted that both α- and β-adrenoceptors are desensitized in hypothermia associated with cardiopulmonary bypass (46).

In view of the findings referred to above, we wanted to further characterize the effects of induced moderate hypothermia on splanchnic blood flow, with particular reference to that of the pancreas and the islets of Langerhans. To further address the issue of whether the nervous system, especially the sympathetic nerves (14), is involved in the hypothermia-induced blood flow changes, we used pretreatment with different pharmacological agents and surgical vagotomy before the induction of hypothermia.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats weighing ∼320 g from a local breeding colony (Biomedical Centre, Uppsala University, Uppsala, Sweden) were used in all experiments. The animals had free access to pelleted rat food (type R3; Ewos, Sollentuna, Sweden) and tap water until the night before the experiments, when food was withdrawn. The rats were anesthetized with an intraperitoneal injection of 40 mg/kg body weight of sodium pentobarbitone (Abbott, Sweden) and were ventilated with a Harvard respirator, according to the International Journal of Laboratory Animal Research recommendations. The respiratory rate was 80 breaths/min, with a tidal volume of 1 ml, and the inspired oxygen concentration was 21%.

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withdrawn. The animals were divided into 12 groups. Two groups received each pretreatment as outlined below, and one group in each pair was subjected to hypothermia, whereas the other group was investigated at normothermia. The experiments were reviewed and approved by the Uppsala municipal court animal research ethics committee.

Surgical preparation and induction of hypothermia. All animals were anesthetized with an intraperitoneal injection of thiobutabarbital (Inactin; Research Biochemicals, Natick, MA; 120 mg/kg body wt) and placed on an operating table with access to thermal pads programmed to maintain the body temperature at 38°C (control animals) or 28°C (hypothermic animals). After tracheotomy, polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and vein. The catheter in the femoral vein was used to continuously infuse saline (6 ml·kg body wt⁻¹·h⁻¹) throughout the experiments, and the aortic catheter was used to monitor mean arterial blood pressure by a transducer (PD5 75/1; Druck, Groby, UK).

Hypothermia was induced by placing plastic gloves filled with ice around the body of the animals. Care was taken to keep the plastic dry at all times. After 10–15 min, the body temperature had dropped to 28°C; this level was maintained by thermal pads preset to this value. Registration of body temperature was made by a thermistor probe inserted into the descending colon. Body temperature was kept at 28°C or >37°C for at least 20 min before blood flow measurements were made.

Pretreatment of animals. Before being subjected to cooling or control procedures, but after induction of anesthesia, the animals were treated according to one of the protocols outlined below. All pharmacological agents (from Sigma Chemicals, St. Louis, MO, unless otherwise stated) were dissolved in saline and given as a bolus intravenous injection (1 ml/kg body wt) after surgical preparation but before commencement of hypothermia induction. Details of protocols are as follows: atropine, a parasympatolytic ACh muscarinic receptor blocker, was given at 10 mg/kg body wt; hexamethionium, which blocks nicotinergic ACh signaling in autonomic ganglia, was given at 10 mg/kg body wt; propranolol, a nonselective β-adrenoceptor antagonist, was given at 10 mg/kg body wt; yohimbine, an α₂-adrenoceptor antagonist, was given at 2.5 mg/kg body wt; and abdominal vagotomy was performed by isolating and dividing the vagus nerves directly against the body in a right angle to the hilar region. Tissue samples were made.

Blood flow measurements. The experiments were performed according to a protocol previously described in detail (24). Briefly, after a stable induction of body temperature had been achieved (see above), 1.5–2.0 × 10⁶ nonradioactive microspheres (E-Z Trac; ITM Products, San Diego, CA) with a mean diameter of 10 μm were injected during 10 s via the catheter placed with its tip in the ascending aorta. Starting at 5 s before the microsphere injection and continuing for a total of 60 s, an arterial blood sample was collected from the catheter in the femoral artery at a rate of ~0.30 ml/min. The exact withdrawal rate was determined in each animal by weighing the sample. After the reference sample was obtained, another blood sample was drawn for measurement of hematocrit, blood glucose, and serum insulin concentrations. After the animals had been killed, the pancreas and adrenal glands, as well as samples from the pylorus, duodenum, ileum, colon, and left kidney, were removed, blotted, and weighed. Samples from the kidney were obtained by cutting a thin slice through the kidney at a right angle to the hilar region. Tissue samples were then treated with a freeze-thawing technique to visualize the microspheres as previously described (23). The blood flow values were calculated according to the following formula: Q_{org} = Q_{ref} × N_{org} / N_{ref}, where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of the reference sample (ml/min), N_{org} is number of microspheres present in the organ, and N_{ref} is number of microspheres in the reference sample. A difference of <10% in blood flow values between the adrenal glands was used to confirm adequate mixing of the spheres in the circulation.

Measurements of blood glucose and serum insulin concentrations. Arterial blood samples were obtained after the reference blood sample was secured; blood glucose concentrations were analyzed with a blood glucose meter (Medisense; Svenska Medisense, Stockholm, Sweden) and serum insulin concentrations with ELISA (rat insulin ELISA; Mercodia, Uppsala, Sweden), with rat insulin (Novo Nordic, Bagsværd, Denmark) as a standard.

Statistical calculations. All values are means ± SE. Probabilities (P) of chance differences between the groups were calculated by Student’s t-test between the normothermic and hypothermic saline-treated animals for each organ. Differences between treatments were tested within either the normothermic or the hypothermic groups for each organ by ANOVA and paired t-tests with Bonferroni correction. Statistical analyses were performed using R version 2.1.0 (49).

RESULTS

Of the 82 animals used in this study, 6 were excluded, 3 because of uneven microsphere distribution and 3 because of uncontrolled shivering during hypothermia. Body temperature was almost exactly 28°C in all animals in the hypothermic group (variation <0.2°C) and 37.5°C (variation <0.3°C) in the normothermic group. Blood glucose concentrations were not affected by hypothermia per se (Table 1). The β-adrenoceptor blocker propranolol increased and vagotomy decreased blood glucose in both normothermic and hypothermic rats. Yohimbine lowered blood glucose in hypothermia but had no effect in normothermia. Neither hexamethionium nor atropine pretreatment affected blood glucose. Serum insulin concentrations Table 1. Physiological characteristics of the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Glucose, mmol/l</th>
<th>Serum Insulin, ng/ml</th>
<th>MAP, mmHg</th>
<th>Hct, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normothermia</td>
<td>5.6±0.3</td>
<td>1.6±0.2</td>
<td>115±3</td>
<td>44.9±0.6</td>
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<tr>
<td>Hypothermia</td>
<td>6.8±0.5</td>
<td>1.3±0.1</td>
<td>110±5</td>
<td>48.3±1.2*</td>
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<tr>
<td>Atropine treatment</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normothermia</td>
<td>6.3±0.1</td>
<td>3.1±0.3</td>
<td>79±3‡</td>
<td>46.2±0.6</td>
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<tr>
<td>Hypothermia</td>
<td>7.3±0.1</td>
<td>1.5±0.1</td>
<td>72±3‡</td>
<td>49.2±0.6</td>
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<tr>
<td>Hexamethonium treatment</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normothermia</td>
<td>5.4±0.2</td>
<td>4.1±0.29†</td>
<td>92±6</td>
<td>42.5±0.3</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>7.4±0.5</td>
<td>1.6±0.17</td>
<td>76±2‡</td>
<td>44.6±0.3‡</td>
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<td>Propranolol treatment</td>
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<td></td>
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<tr>
<td>Normothermia</td>
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<td>1.78±0.21</td>
<td>91±3‡</td>
<td>45.0±0.4</td>
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<td>1.77±0.18</td>
<td>81±10†</td>
<td>49.8±0.6</td>
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<td>Vagotomy</td>
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<td></td>
</tr>
<tr>
<td>Normothermia</td>
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<td>3.45±0.76†</td>
<td>111±6</td>
<td>44.9±0.8</td>
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<td>Yohimbine treatment</td>
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<td>Normothermia</td>
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<tr>
<td>Hypothermia</td>
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<td>2.26±0.29</td>
<td>124±2</td>
<td>45.6±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 or 8 experiments. Shown are blood glucose, serum insulin, mean arterial blood pressure (MAP), and hematocrit (Hct) values during normothermia (37.5°C) or hypothermia (28°C) in animals pretreated with an intravenous injection of either saline, atropine, hexamethonium, propranolol, or yohimbine or with surgical subdiaphragmatic vagotomy. *P < 0.05 between saline-treated normothermic and hypothermic animals. †P < 0.05 compared with saline-treated normothermic control animals. ‡P < 0.05 compared with saline-treated hypothermic controls.
remained unchanged after hypothermia (Table 1). Hexamethonium increased serum insulin in normothermic but had no effect in hypothermic rats.

Mean arterial blood pressure was not affected by hypothermia but decreased after pretreatment with atropine, hexamethonium, or propranolol in normothermic and in hypothermic rats (Table 1). Vagotomy or yohimbine elicited no change in mean arterial blood pressure. Hematocrit values were increased in all hypothermic animals, except those pretreated with hexamethonium, where the value was lower (Table 1).

Pancreatic blood flow increased during hypothermia (Fig. 1) and so did islet blood flow (Fig. 2). Pancreatic blood flow was otherwise affected only during propranolol pretreatment of hypothermic rats, where a diminished blood flow was seen compared with that shown in hypothermic control rats (Fig. 1). Islet blood flow was increased by propranolol and hexamethonium pretreatment in normothermic rats (Fig. 2). Administration of hexamethonium during hypothermia further augmented the islet blood flow increase, whereas no increase in islet blood flow was seen in hypothermic rats pretreated with atropine, propranolol, yohimbine, or vagotomy (Fig. 2).

Pyloric blood flow increased during hypothermia (Fig. 3), a phenomenon that was prevented by all experimental interventions. However, both hexamethonium administration and vagotomy markedly decreased pyloric blood flow in both normo- and hypothermic rats. Duodenal blood flow was unaffected by hypothermia and showed no reaction to pharmacological interventions, whereas vagotomy decreased blood flow in hypothermia (Table 2). Ileum and colon showed no changes in blood flow during hypothermia, and their blood perfusion was unaffected by any of the given treatments (Table 2).

Hypothermic control animals had a lower renal blood perfusion than the corresponding control rats (Fig. 4). Propranolol administration had no effect in normothermic rats and did not affect the decrease in renal blood flow during hypothermia. Hypothermia decreased adrenal blood flow in all groups. In hexamethonium-treated rats, there was no discernable difference between blood flow in the normothermic and hypothermic rats (Table 2).

DISCUSSION

We found that moderate hypothermia increased pyloric, total pancreatic, and islet blood flow, whereas renal and adrenal blood flows were decreased and the other splanchnic organs had an unaffected blood flow.

The nonspecific β-adrenoceptor antagonist propranolol increased islet blood flow in normothermic animals compared with that shown in normothermic controls, and the 2-adrenoceptor antagonist yohimbine produced a higher islet blood flow in normothermia, although the difference did not attain statistical significance. Hexamethonium also produced a strong increase of islet blood perfusion in normothermia in contrast to our previous results in other rat strains (21). This may, at least partially, reflect the use of different rat strains. Total pancreatic blood flow in normothermia, on the other hand, was unaffected by any of the pretreatments given.

All pretreatments, besides hexamethonium, prevented the hypothermia-induced increase in total pancreatic blood flow. The hypothermia-induced islet blood flow increase was similarly prevented by all pretreatments, also in this case with the exception of hexamethonium. It should be noted, however, that especially the islet blood flow is already increased by several of the pretreatments given, and increased hypothermia-induced
sympathetic stimulation may not be able to further add to this. With regard to total pancreatic blood flow, this may be the case in propranolol-treated rats, since the flow value was decreased compared with that shown in control hypothermic rats. In view of this, it seems as if both the parasympathetic and sympathetic nervous system may be involved in the increase in pancreatic and islet blood flow observed during moderate hypothermia. Thus it is a truly multifactorial response that is elicited in these animals.

In view of the blood flow responses to inhibition of sympathetic or parasympathetic nerves, it is puzzling that the ganglionic blocker hexamethonium actually increases the blood perfusion. It can be speculated that, during these functionally denervated conditions, a shift in the balance between locally produced vasoconstrictors and vasodilators occurs. We have previously shown that pancreatic islets are extremely sensitive to both nitric oxide (48) and endothelin-1 (30), and to what extent hypothermia affects the effects of these substances certainly merits further investigation.

With regard to the blood flow to other parts of the gastrointestinal canal, pyloric blood flow was increased by hypothermia, but no change was seen in the duodenum, ileum, or colon. This is in contrast to some other studies in which decreased (13, 42) or increased (39) mucosal intestinal or gastric blood flows, respectively, were observed during hypothermia. However, other studies have demonstrated an unchanged mucosal blood perfusion (27). The colon and ileum responded little to pretreatment, whereas the duodenum and pylorus both showed a decreased blood flow after vagotomy and the pylorus responded strongly also to hexamethonium, which is in line with earlier results (17). Thus the major effects of hypothermia seem to be on the pylorus and pancreatic islets. From a teleological point of view, this can be explained by the fact that ingestion of cold food should still be able to increase gastric and islet activity to facilitate digestion and thereby also increase blood flow. Pyloric blood perfusion seems to be increased mainly through the parasympathetic system, whereas islet blood flow is stimulated through the sympathetic system.

The blood glucose concentrations were only marginally affected by any of the treatments given and is unlikely to affect any of the observed blood flow values (20). There is a discrepancy between the changes in blood glucose and serum insulin in hexamethonium-pretreated hypothermic rats, where insulin increases and blood glucose is unchanged. This is likely to reflect changes in insulin sensitivity caused by the increased

![Graph](http://ajpregu.physiology.org/)
sympathetic activity or a direct release of glucose from skeletal muscle and/or liver.

Reduced basal insulin secretion in combination with blunted glucose-induced insulin secretion and constant or increased glucagon secretion leads to glucose intolerance in hypothermia (19, 32). The same pattern is observed in hibernating squirrels, where somatostatin and pancreatic polypeptide has also been shown to be decreased (4). Somatostatin is known to inhibit both glucagon and insulin release (36, 52) and shows equal efficiency during shivering thermogenesis and control conditions in dogs. The combined evidence of increased glucagon and decreased insulin in rats in combination with decreased somatostatin in other models leads us to believe that pancreatic somatostatin secretion is decreased and that the imbalance between induced insulin and glucagon secretion seen in hypothermia and the slight decrease in insulin that we show in nonstimulated conditions are not somatostatin related. This is consistent with our previous finding (11) that the somatostatin analog octreotide decreases islet blood flow, whereas the present results show an increase in hypothermia.

The present findings of a decreased renal blood flow during moderate hypothermia confirm previous results (9, 10). In the present study, pretreatment with hexamethonium abolished the downregulation of renal blood flow in hypothermia, suggesting that the nervous system is involved in this response. However, the results are somewhat ambiguous because the hexamethonium-treated normothermic rats showed a decreased blood flow at a level comparable with the saline-treated hypothermic animals without attaining statistical significance. Propranolol elicited a renal blood flow decrease in hypothermic animals beyond what was seen in saline-treated animals. This is compatible with recent results showing a β-receptor-mediated dilatation in isolated renal afferent arterioles (51) and that they may be sensitized in hypothermia (54). Most of these effects are likely to emanate from circulating catecholamines, since denervation does not affect hypothermia-induced decreases in renal blood flow (10). Indeed, previous studies in human kidneys have shown that intrarenal α-adrenoceptors mediate a tonic renal vasoconstriction (1, 15, 35).

The adrenal glands demonstrated a marked attenuation of blood flow during hypothermia, and this response was unaffected by administration of any of the tested substances. However, propranolol markedly increased adrenal blood flow in normothermic rats. The regulation of adrenal medullary and cortical blood flow is complex and mediated through both neural and metabolic mechanisms (6). The contribution of various transmitters in this context is controversial, and conflicting results have been presented (3, 6). The results in the present study suggest that β-adrenoceptors are involved in this process, but this awaits further experimental confirmation.

Blood pressure was, as expected, lowered by propranolol, atropine, and hexamethonium (33). However, the changes in the vascular conductance of the studied organs mimicked those of the measured blood flow values (data not shown).

Hematocrit affects blood viscosity and is thereby an important factor in determining blood flow. The effect seen in the hypothermic animals in this study is in agreement with our earlier results (9, 10). We have previously calculated that this difference causes a 10% increase in viscosity (8), which would have minor effects on the results of the present study. Hexamethonium-pretreated rats did not demonstrate any increase in hematocrit, which may be due to an effect on the precapillary vasoconstriction.

We conclude that moderate hypothermia elicits complex and largely organ-specific circulatory changes, with increased perfusion noted in the pylorus and in the whole pancreas and the pancreatic islets. The pancreatic islets maintain their high blood perfusion through mechanisms involving both sympathetic and parasympathetic mediators, whereas the increased pyloric blood flow is mediated through parasympathetic mechanisms. Renal blood flow was decreased, and we have provided evidence that this can be prevented by ganglionic blockade and that it is influenced by β-adrenoceptors.

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