GLUCOSE UPTAKE
IN
SKELETAL MUSCLE

By

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A thesis submitted in requirement for the degree of
Doctor of Philosophy

Division of Biochemistry
University of Tasmania
2006
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STATEMENT

The work in this thesis has been undertaken exclusively for the use of a Ph.D. in the area of Biochemistry, and has not been used for any other higher degree or graduate diploma in any university. All written and experimental work is my own, except that which has been referenced accordingly.

Cathryn Kolka

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ABSTRACT

Glucose uptake occurs in skeletal muscle under basal conditions, and increases in response to stimuli such as insulin and exercise. Exercise is known to increase blood flow, and it appears that insulin has similar hemodynamic effects, including increased blood flow and capillary recruitment, which can modify the amount of glucose uptake occurring under each condition. Here we study factors affecting both basal and stimulated myocyte glucose uptake, with a particular focus on vasoactive agents.

Insulin stimulates the release of endothelin-1 (ET-1), a potent vasoconstrictor, from endothelial cells in culture. As yet it is unknown whether ET-1 is a type A (causing nutritive perfusion) or a type B (non-nutritive) vasoconstrictor, so here we use the pump-perfused rat hindlimb to characterize the distribution effects of ET-1. We show that ET-1 causes a type A vasoconstriction, stimulating basal metabolism at low doses, while at high doses the distribution of flow changes to become non-nutritive, inhibitory to metabolism. As a general vasodilator prevents both metabolic and hemodynamic effects, the effects on metabolism are due to the redistribution of flow. These redistribution effects are confirmed by the ability of high dose ET-1 to decrease aerobic tension development in the contracting hindlimb, and by the ability of low dose ET-1 to increase the interstitial glucose concentration.

Given this understanding of the effects of ET-1 alone, we can investigate the interactions between ET-1 and insulin. In the perfused rat hindlimb, insulin has not been observed to have any vasodilatory effect, whereas here for the first time insulin appears to have vasodilator-like actions against ET-1 mediated vasoconstriction. Also, the redistribution of flow by ET-1 does not appear to alter the metabolic effect of insulin to cause glucose uptake at either dose of ET-1 used.

Nitric Oxide (NO) is thought to be the mechanism by which insulin causes vasodilation in muscle. A previous study has shown that methacholine (MC), by increasing NO, was able to augment insulin-mediated glucose uptake and capillary recruitment, while other NO donors were unable to do so. Here we show that, at the dose used to increase glucose uptake in the previous study, MC has only a vasodilatory effect, and no direct effect on glucose uptake, in the perfused rat hindlimb. At higher doses, an effect on glucose uptake can be observed. This means that the increase in capillary recruitment by MC was responsible for the elevated insulin-mediated glucose uptake, and there was no direct effect of MC on glucose uptake.

A recent publication suggested that the Na\(^+\)-D-glucose cotransporter (SGLT1) was essential for insulin-mediated glucose uptake, although not required for basal glucose uptake. The implications of this detract from our proposed role of blood flow redistribution in insulin action. In attempting to reproduce these results in the perfused rat hindlimb we found that SGLT1 is not required for insulin-mediated glucose uptake, and confirmed this using a low sodium buffer, which would also inhibit the transporter. We conclude that SGLT1 is not required for insulin-mediated glucose uptake.

Our results therefore suggest that complex interactions are involved in insulin action, some of which involve hemodynamic actions that are capable of altering insulin-mediated glucose uptake, and others in which insulin itself can limit the action of other vasomodulators, such as ET-1. It is apparent, however that SGLT1 in the endothelium may not be necessary for the metabolic effects of insulin, and that blood flow distribution, or capillary recruitment is therefore of great importance in delivering glucose to myocytes.
ACKNOWLEDGMENTS

I wish to thank Prof Clark, for his guidance and supervision during the last four years, without him I am sure my work would not have gone as well, and the ‘Steve’s’ (Rattigan and Richards), for all their help and suggestions along the way.

I would also like to thank many past and present members of the muscle research group, including Cate, Hema, Lei, Georgie, Maree, Phil, Amanda, John, Carole, Merren and Geoffrey, for making my time here enjoyable. An extra special thanks to Renee and Eloise – for your friendship and help along the way.

Also thanks to those hard workers in the animal house, especially Marcus and Murray, and all the friends I have made in the rest of the building as well, particularly those in the MBU.

Thanks also go to my family, friends and to Justin for their love, support and friendship.
# Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A-V</td>
<td>arterio-venous</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>AII</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>e</td>
<td>extensor digitorum longus</td>
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<tr>
<td>EDRF</td>
<td>Endothelium-derived relaxing factor</td>
</tr>
<tr>
<td>ET</td>
<td>Endothelin</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
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<td>ETA</td>
<td>Endothelin receptor type A</td>
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<tr>
<td>ETB</td>
<td>Endothelin receptor type B</td>
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<td>GLUT</td>
<td>facilitative glucose transporters</td>
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<tr>
<td>Ins</td>
<td>Insulin</td>
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<tr>
<td>L-NAME</td>
<td>Nitro-L-Arginine Methyl Ester</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>mono-methyl nitro-L-arginine</td>
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<tr>
<td>MC</td>
<td>Methacholine</td>
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<td>NE</td>
<td>Norepinephrine</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<td>NOS</td>
<td>Nitric Oxide Synthase</td>
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<td>eNOS</td>
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<tr>
<td>Nox</td>
<td>end-products of oxidized NO</td>
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<td>plantarus</td>
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<tr>
<td>r</td>
<td>red gastrocnemius</td>
</tr>
<tr>
<td>R’g</td>
<td>Rate of glucose uptake</td>
</tr>
<tr>
<td>s</td>
<td>soleus</td>
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<td>Abbreviation</td>
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<tr>
<td>SGLT1</td>
<td>sodium-glucose co-transporter</td>
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<td>sodium nitroprusside</td>
</tr>
<tr>
<td>t</td>
<td>tibialis</td>
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</tr>
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<td>white gastrocnemius</td>
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<td>1-MX</td>
<td>1-methylxanthine</td>
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<td>5HT</td>
<td>serotonin</td>
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PREFACE

Some of the data presented in this thesis has been published or presented at scientific meetings and has been listed below.

Publications arising directly from this thesis

Kolka CM, Rattigan S, Richards S, Clark MG. Metabolic and vascular actions of endothelin-1 are inhibited by insulin-mediated vasodilation in perfused rat hindlimb muscle. British Journal of Pharmacology. 2005 May 16


Kolka CM, Rattigan S, Richards S, Clark MG. Reduced exercise capacity in hypertension: a consequence of endothelin-mediated functional shunting. (Manuscript in preparation.)

Other publications

Wallis MG, Smith ME, Kolka CM, Zhang L, Richards SM, Rattigan S, Clark MG. Acute glucosamine-induced insulin resistance in muscle in vivo is associated with impaired capillary recruitment. Diabetologia. 2005
Posters at scientific meetings


Endothelin-1 as a messenger for insulin has both stimulatory and inhibitory effects on perfused muscle metabolism via its vascular actions. Kolka CM, Rattigan S, Richards S, Clark MG.


Endothelin-1 via its vascular actions in muscle can be either supportive or antagonistic of insulin. Kolka CM, Rattigan S, Richards S, Clark MG.

European Association for the Study of Diabetes annual meeting, Sept 11th-14th 2005, Athens, Greece.

Endothelin-1 vascular and resultant metabolic actions in perfused rat hindlimb are opposed by insulin. Kolka CM, Rattigan S, Richards SM, Clark MG.

Glucosamine induces acute insulin resistance in muscle in vivo associated with impaired capillary recruitment. Clark MG, Wallis MG, Smith ME, Kolka CM, Zhang L, Richards SM, Rattigan S.

Heart Foundation Conference and Scientific Meeting, March 23rd-25th 2006, Sydney Australia

Reduced exercise capacity in hypertension: A consequence of endothelin-mediated functional shunting of blood flow in muscle. Kolka CM, Rattigan S, Richards SM, Clark MG.