EVIDENCE FOR AN INVOLVEMENT OF KININ LIBERATION IN THE PRIMING ACTION OF INSULIN ON GLUCOSE UPTAKE INTO SKELETAL MUSCLE

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Received 26 October 1978

1. Introduction

In order to explain the insulin-independent work-induced acceleration of glucose uptake into skeletal muscle [1,2], a humoral factor was postulated which was supposed to be released by the tissue under these circumstances [3]. Recently, kinins liberated from kininogen during muscle work were recognized as possible candidates for the requested factor [4]. This was emphasized by almost identical results obtained during muscle hypoxia [5] and by the finding that the molecular weight estimated for the factor [6] was almost identical to that of kinins.

Since kinin liberation was also supposed to be involved in insulin action on glucose uptake into resting muscle [7], it appeared of interest to investigate whether the well-known permissive action of insulin on glucose uptake during muscle work [8] will also be impaired when kinin liberation from kininogen is prevented.

2. Materials and methods

Healthy volunteers (n = 8,4,4) were recruited from medical students. They were informed about the aim and the possible risks of the study and gave their consent according to the Code of Ethics of the World Medical Association [9]. Physical examination as well as laboratory tests excluded internal diseases. The three groups were well comparable as to their age, height and weight. All subjects were fasted overnight (15 h) and received no drugs or premedication.

Under sterile conditions and local anaesthesia an Abbocath-T-needle (20-G, Abbot Laboratories, North Chicago, IL 60064) was inserted into a brachial artery and was flushed continuously with 154 mM NaCl (0.2 ml/min) and heparin at a low enough rate (0.1 U/kg × min) to exclude a significant effect on lipoprotein lipase activity [10]. Then, one of the cubital veins apparently draining deep forearm tissue areas was cannulated at the other arm, and a teflon catheter (Code 11512, Vygon) inserted retrograde as deep as possible. In addition, brachial artery of this arm was cannulated with a Abbocath-T needle (see above) and flushed continuously with 154 mM NaCl (0.2 ml/min) without heparin.

After an accommodation period of 15 min arterial and deep-venous blood samples were collected simultaneously throughout a 10 min basal period for chemical analysis at 5 min intervals.

Then, in 8 subjects, during intrabrachial arterial infusion of 154 mM NaCl, rhythmic–isometric exercise, standardized by a special ergometer [11] was performed for 3 min (0.82 W). Blood samples were obtained at 2.5 min and 3 min during work load and 4 min afterwards. During the whole test period muscle blood flow was registered continuously [12] after intramuscular injection of 0.3 mCi ^{133}Xe dissolved in 0.1 ml saline, counting its washout by a scintillation counter (Model 302, Packard).

In 4 other subjects, after collecting basal blood samples, 10 min before starting identical forearm work, 150 μU insulin/kg × min (Altinsulin Hoechst CS®, Hoechst AG, Frankfurt) dissolved in 154 mM NaCl were infused into the brachial artery of the working arm for the whole following test period at 0.2 ml/min. 3 min after starting insulin infusion

Table 1

Forearm blood flow and arterial–deep-venous differences of substrates at rest, during rhythmic–isometric forearm exercise and recovery during intraarterial infusion of 154 mM saline (C), of insulin (I) and of insulin plus intravenous infusion of kallikrein–trypsin inhibitor (II)

		Rest		Work		Recovery
		Basal	Insulin	2.5 min	3 min	4 min
Muscle blood flow[d]	C	1.9 ± 0.1		14.2 ± 1.6[a]		2.0 ± 0.1
	I	2.0 ± 0.2		15.2 ± 2.3[a]		2.1 ± 0.1
	II	1.8 ± 0.1		6.8 ± 1.7[a,b,c]		2.0 ± 0.1
Oxygen[e]	C	7.9 ± 0.8	–	14.4 ± 0.5[a]	14.7 ± 0.4[a]	7.7 ± 1.2
	I	8.7 ± 0.7	7.8 ± 1.4	14.1 ± 0.6[a]	14.4 ± 0.7[a]	7.4 ± 1.7
	II	7.3 ± 0.7	6.7 ± 0.7	14.3 ± 0.6[a]	13.9 ± 0.7[a]	8.5 ± 0.7[a]
Glucose[f]	C	32.4 ± 4.8	–	40.0 ± 5.3[a]	37.5 ± 4.5[a]	35.6 ± 5.8
	I	29.3 ± 4.8	50.8 ± 4.5[a]	56.3 ± 2.4[a,b]	52.8 ± 3.8[a,b]	64.0 ± 8.5[a,b]
	II	27.3 ± 8.1	38.0 ± 9.9	40.5 ± 6.8[a,c]	35.3 ± 6.9[a,c]	49.8 ± 16.8[a]
Lactate[f]	C	−15.1 ± 1.7	–	−40.3 ± 5.3[a]	−37.3 ± 7.3[a]	−22.3 ± 3.9[a]
	I	−17.4 ± 4.5	−13.5 ± 1.9	−20.4 ± 4.2[b]	−18.2 ± 2.5[b]	−19.9 ± 4.0
	II	−10.7 ± 1.7	− 8.9 ± 3.0	−21.5 ± 11.0	−18.8 ± 8.8	− 7.0 ± 3.5[b,c]

[a] Indicates significant difference to basal (paired *t* - test), [b] as compared to C (unpaired *t* - test) and [c] as compared to I (unpaired *t* - test), further details in section 2

The values represent the mean ± SEM of 8 (C), 4 (I) and 4 (II) healthy volunteers in ml/100 g muscle and min[d], in ml/100 ml[e] and in μmol/100 ml[f]. – Indicates release

1 mg glucose/kg × min (0.555 M sterile glucose solution) was applied into an anticubital vein of the non-working arm in orde to maintain constant arterial glucose level [7].

Another 4 subjects received first after the basal period infusion of a kallikrein–trypsin inhibitor (Trasylol®, containing 500 000 kallikrein inhibitor units in 50 ml isotonic solution, Bayer AG, Leverkusen) into an anticubital vein of the non-working arm for the whole following test period (500 000 kallikrein inhibitor units within 15 min and then 12 500 kIU/min. At min 10 after starting inhibitor infusion identical infusion of insulin into the brachial artery of working arm and 3 min later infusion of glucose into an anticubital vein of the non-working arm was applied.

In both these groups receiving insulin or inhibitor plus insulin, one arterial and deep-venous blood sample was taken 8 min after starting insulin infusion. Forearm work thereafter started at min 10 and blood sampling was done as indicated in the control group.

Analyses of substrates, insulin and oxygen were performed as in [7,13]. Standard statistical methods were employed [14] using the Student's *t*-test for paired and unpaired samples when applicable.

3. Results

Arterial concentrations of substrates did not change during the whole test period and were almost identical in all the groups:

Glucose: control [C]: 4.74 ± 0.09 mmol/l; insulin [J]: 4.59 ± 0.13 mmol/l; insulin plus inhibitor [JJ]: 4.92 ± 0.05 mmol/l.

Lactate: C: 0.41 ± 0.03 mmol/l; J: 0.33 ± 0.04; JJ: 0.41 ± 0.07.

β-hydroxybutyrate: C: 0.15 ± 0.03 mmol/l; J: 0.22 ± 0.04; JJ: 0.10 ± 0.03.

8 min after starting intra-arterial infusion of insulin before work identical deep-venous concentrations of insulin were measured in both groups (J: 100.0 ± 43.8, JJ: 139.8 ± 37.0 μU/ml). At min 2.5 during work a slight decrease was found (J: 79.5 ± 24.6, JJ: 94.5 ± 29.4 μU/ml).

The mean values for blood flow and for arterial–deep-venous differences of glucose, lactate and oxygen are indicated in the table, the values for muscle uptake of glucose in the figure.

Muscle release of lactate was found to be identical in the 3 groups at rest and during infusion of insulin with and without the inhibitor. However, during work

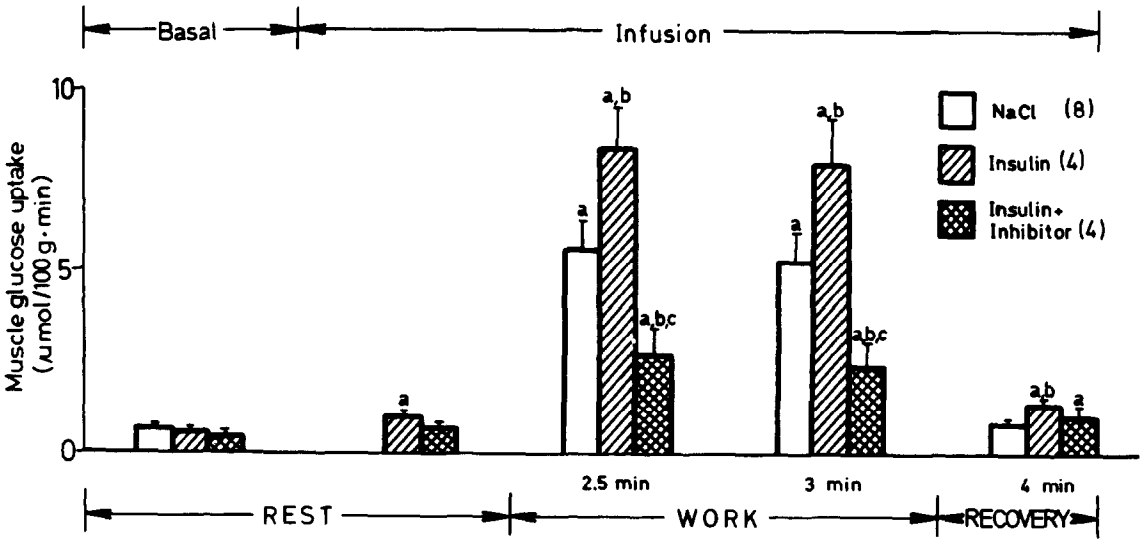

Fig.1. Glucose uptake into skeletal muscle of forearm. The values were calculated from the arterial-deep-venous glucose differences and the corresponding blood flow rates and represent the mean ± SEM. Insulin was infused into the brachial artery, the inhibitor (Trasylol®) into an antecubital vein. (a) Indicates significant difference to basal (paired *t*-test); (b) to control (unpaired *t*-test) and (c) to insulin (unpaired *t*-test). Further details under section 2.

muscle lactate release was enhanced up to −4.83 ± 0.67 μmol/100 g × min in C and only up to −2.72 ± 0.42 in J and to −1.45 ± 0.71 in JJ. During recovery muscle lactate release was found to be lessened in JJ (−0.14 ± 0.07) as compared to C or J (−0.44 ± 0.08, −0.42 ± 0.08).

Muscle uptake of oxygen was identical during rest and recovery in the 3 groups. During work, muscle oxygen consumption increased to the same extent to 2.08 ± 0.21 ml/100 g × min in C and to 1.85 ± 0.27 in J, but only up to 0.87 ± 0.09 ml/100 g × min in JJ.

4. Discussion

The aim of this study was to investigate whether the well-known permissive action of insulin on accelerated glucose uptake into skeletal muscle during work load [8] could be impaired when liberation of kinins from kininogen was prevented by the infusion of a kallikrein–trypsin inhibitor (see section 2).

The model employed was previously proven to be suitable for this purpose [15]. Although the ^{133}Xe-injection technique employed for the measurement of blood flow is encumbered by several difficulties, it is supposed to yield representative increases of muscle blood flow during muscle work [16]. According to the moderate work load, glucose uptake was already increased at 2.5 min and 3 min, and the values obtained are in good agreement with those in [17].

Insulin was used at a dose which was already observed earlier to give only small changes of glucose metabolism in the resting human forearm [18,19]. As had been found in the in vitro model [8] the acceleration of glucose uptake during muscle work was further increased significantly during insulin infusion. Lesser lactate release of working muscle under the influence of insulin was most probably due to increased pyruvate oxidation resulting from inhibition of muscle lipolysis [20] and consequently decreased availability of endogenous free fatty acids for oxidation. Both, the effect of work and the permissive action of insulin on glucose uptake were almost completely abolished by the kallikrein inhibitor employed. Identical deep-venous insulin concentrations obtained behind the muscle in both groups proved identical hormone drive, identical substrate supply also comparable metabolic conditions (see section 3). Since the work performed was identical in all the cases, reduced oxygen utilization during the infusion of the inhibitor was supposed to be rather a consequence of prevented glucose uptake especially considering the impaired availability of other substrates during insulin infusion.

The inhibition of liberation of kinins from kininogen by a kallikrein inhibitor had been found to reduce glucose uptake into muscle during work [4]. That this effect was really due to inhibition of the activity of the enzyme kallikrein was very probable since substitution of bradykinin into the brachial artery was able to restore the normal response during work load. That the reduced glucose uptake observed was not a consequence of reduced oxidative metabolism was furthermore strengthened by earlier data also showing reduction of insulin-induced acceleration of glucose uptake into the resting human forearm by the inhibitor employed [7]. That this was due to prevented liberation of kinins from kininogen was underlined by the finding that inhibition of prostaglandin biosynthesis which had been found to translate the action of kinins, prevented the action of insulin on glucose uptake to a similar extent [21]. Nevertheless, it has to be discussed finally that also other kinds of proteases could have been influenced by the inhibitor since trasylol is no specific kallikrein inhibitor [22]. However, since there is no clear evidence so far that proteases are involved in insulin action [23] inhibition of the activity of the kininogenase kallikrein seems to be the most probable reason for the observed effect.

Acknowledgements

This work is dedicated to Professor Dr Dr E. K. Frey and Professor Dr E. Werle. The authors are indebted to Professor Dr Kraut and Professor Dr H. Mehnert for valuable discussions and support. They wish to acknowledge the excellent technical assistance of E. A. Bauer, A. Bammert, E. Maerker, H. Kirschner and G. Drotleff. This work was supported by a grant of the Sonderforschungsbereich 51 of the Deutsche Forschungsgemeinschaft.

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