EVIDENCE FOR A PARTICIPATION OF THE KALLIKREIN–KININ SYSTEM IN THE REGULATION OF MUSCLE METABOLISM DURING MUSCULAR WORK

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1. Introduction

For 80 years it has been well known that muscular work stimulates muscular glucose uptake [1]. As this phenomenon was observed even at low insulin levels local liberation of a 'muscular activity factor' was postulated [2]. Recently, it became evident that small insulin concentrations are indispensable for the increase of muscular glucose entry to occur during exercise [3].

In this paper data will be presented which show that the kallikrein–kinin system is also involved in the adaptation of muscle metabolism to muscular exercise.

2. Materials and methods

Twelve healthy volunteers were recruited from medical students. All were informed about the aims and the risks of the study and gave their consent. Physical examinations as well as laboratory tests excluded internal diseases. After an overnight fast in a recumbent position one of the cubital veins apparently draining deep tissue of the forearm was canulated under sterile conditions. After local anaesthesia a Teflon-catheter (Code 11512, Vygon, France) was inserted retrograde as deep as possible. Then, a Cournand-needle (PE 160, Kifa, Sveden) was inserted into the right femoral artery and a thin Luer-Lok canula (A 150, Henke-Sass Wolf, FRG) into the brachial artery and flushed continously with physiological saline (0.2 ml/min).

Blood samples were collected simultaneously from the femoral artery and deep antecubital veins throughout a 15 min basal period for chemical analysis. Glucose was determined enzymatically with the precision given elsewhere [5]. Then, in 4 subjects, during infusion of physiological saline, rhythmic-isometric exercise, standardized by a special ergometer [6] was performed by the forearm for 3 min.

Blood samples were obtained at 2.5 min and 3 min during the work load and 2 min and 4 min afterwards. During the whole test period muscle blood flow was registered continuously [7] after intramuscular injection of 0.3 mCi ^{133}Xe dissolved in 0.1 ml saline, counting its wash-out by a scintillation counter (Model 302, Packard, USA). In 4 subjects, under identical forearm work, a kallikrein–trypsininhibitor (Trasylol, Bayer AG, FRG) was infused intravenously at a rate of 500 000 Kallikrein-inhibitor-units (KIU)/10 min. Another 4 subjects received, in addition to trasylol, bradykinin (synth. bradykinin, Sandoz AG, FRG) into the brachial artery, at an infusion rate of 13.3 ng/min. Blood sampling and exercise were done as indicated above. Standard statistical methods were employed [8] using the Student's *t*-test for paired and unpaired samples when applicable.

All values are given as the means with the standard error of the mean (SEM).

3. Results and discussion

If, as in the present study, the forearm has to perform rhythmic-isometric exercise while the whole body is in a recumbent position, arterial concentrations of hormones and substrates are maintained and local regulation is necessary to support working muscles with oxygen and substrates. The arterial con-

centrations of the substrates analyzed were within the expected ranges [6] and did not change during the exercise performed. Since muscular oxygen extraction capacity is limited, increase of muscular oxygen uptake during muscular work and consequently substrate combustion is preferably dependent on the adaptation of local muscular blood flow (for review see [9]). Acceleration of local perfusion is suggested to be achieved by liberation of an unknown humoral factor which initiates local dilatation of the supporting capillary vessels (for review see [10]). In the present working model under rhythmic-isometric forearm exercise (table 1) muscular blood flow was increased 9-fold ($p < 0.0125$) and oxygen uptake almost 15-fold ($p < 0.005$). Basal values of substrate balances and blood flow were in good agreement with those from earlier studies [6,7].

It is a well known fact that muscular glucose uptake is limited by the rate of glucose entry at the cell surface which can only be accelerated by insulin and muscular work [2,11]. Accordingly, during the work load, glucose uptake (fig.1) was not only

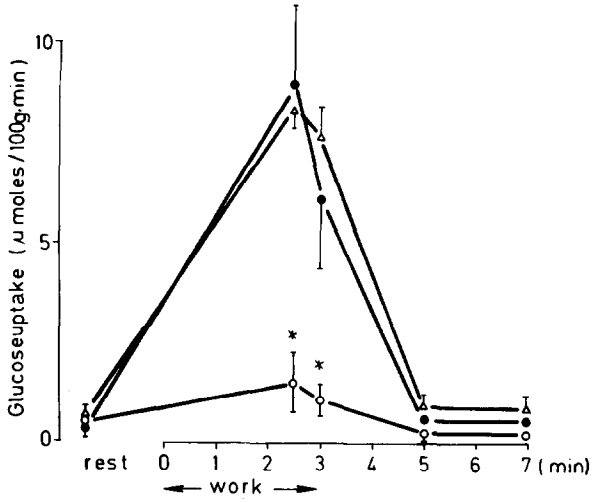

Fig.1. Glucose uptake of the working human forearm during intravenous infusion of physiological saline (●——●), of the kallikrein–kinin inhibitor (○——○) and additional brachial arterial infusion of bradykinin (△——△). Values are indicated as the mean ± SEM of 4 volunteers in each case. (*) Means significant difference as compared to the infusion of physiological saline (●——●) and of the inhibitor and additional bradykinin (△——△) ($p < 0.025$, unpaired t-test). Details under Materials and methods.

enhanced by the acceleration of blood flow (table 1) but also by a significant enlargement of muscular glucose extraction ($p < 0.0125$). Simultaneously muscular lactate output rose almost 40-fold ($p < 0.005$) and acetoacetate uptake maintained.

Since the acceleration of glucose transport during muscular work occurs at low insulin levels, local liberation of a 'muscular activity factor' has been postulated [2] which is responsible for the insulin-independent acceleration of glucose entry. Such a factor would be of special significance if it were to control local muscular blood flow and glucose entry into the muscle cell at the same time.

It was therefore of interest to note that, during identical work load, acceleration of blood flow and increase of glucose extraction were reduced simultaneously when kinin-release from kininogen was inhibited by the application of the kallikrein–trypsin inhibitor.

Under these conditions muscular blood flow increased only 5-fold, consequently limiting muscular oxygen uptake since muscular oxygen extraction was maximally enhanced (table 1). Muscular glucose extraction was even markedly reduced as compared to its basal value ($p < 0.05$) and calculated glucose uptake (fig.1) enhanced only 4-fold revealing a significant difference as compared to the 15-fold increase during saline infusion ($p < 0.005$). Correspondingly, lactate output was also diminished significantly ($p < 0.025$).

If a factor would have maintained to control glucose entry at the cell surface separately, an increase of muscular glucose extraction should have been observed. That glucose, as a substrate for energy demand of the muscle cell was lacking at the end of exercise, might be indicated by simultaneous doubling of muscular acetoacetate uptake as compared to the controls ($p < 0.025$) (table 1). Though, besides the kallikreinkinin system, several proteinases could have been influenced by the inhibitor, it seemed very likely to assume that kinins may be involved in the regulation of local muscular blood flow and glucose transport during muscular work. This view was strongly supported by the finding that small amounts of bradykinin applied simultaneously with the inhibitor did completely restore the muscles' metabolic response to the standard work (table 1, fig.1).

That the kinin did not restore glucose metabolism

Table 1
Effect of rhythmic-isometric forearm exercise on muscle blood flow and forearm deep venous balance of substrates during intravenous infusion of saline (I), of kallikrein–trypsin inhibitor (II) and of kallikrein–trypsin inhibitor plus intrabrachial bradykinin (III)

		Rest	Exercise		Recovery	
			2.5 min	3 min	2 min	4 min
Muscular blood flow[d]	I	1.7 ± 0.1		15.1 ± 3.2[a]	1.9 ± 0.3	
	II	1.7 ± 0.3		8.8 ± 2.0[a,b]	1.8 ± 0.2	
	III	1.9 ± 0.4		15.3 ± 1.8[a,c]	2.2 ± 0.4	
Oxygen[e]	I	9.0 ± 0.9	13.6 ± 0.4[a]	13.0 ± 0.7[a]	10.7 ± 0.6	9.0 ± 0.5
	II	8.9 ± 0.7	13.2 ± 0.4[a]	13.4 ± 0.4[a]	11.4 ± 0.9[a]	11.2 ± 0.7[a,b]
	III	8.3 ± 0.4	12.6 ± 0.9[a]	12.8 ± 0.8[a]	8.2 ± 0.9[c]	7.9 ± 0.7[c]
Glucose[f]	I	21.0 ± 2.3	51.3 ± 8.2[a]	36.3 ± 5.0[a]	30.5 ± 5.7	29.3 ± 7.0
	II	26.8 ± 6.3	15.3 ± 4.1[b]	11.0 ± 2.3[a,b]	13.0 ± 2.3[a,b]	8.5 ± 2.2[a,b]
	III	32.5 ± 5.5	58.8 ± 7.3[a,c]	52.8 ± 4.7[a,c]	42.8 ± 2.7[a,c]	39.3 ± 2.8[c]
Lactate[f]	I	−9.8 ± 2.3	−52.3 ± 19.2[a]	−47.5 ± 18.8[a]	−41.8 ± 15.8[a]	−29.5 ± 14.4
	II	−9.5 ± 1.7	−32.3 ± 8.0[a]	−26.3 ± 8.8[a]	−23.3 ± 8.6	−21.3 ± 11.3
	III	−7.3 ± 2.8	−64.5 ± 17.9[a]	−50.3 ± 17.5[a]	−46.0 ± 17.3[a]	−26.3 ± 8.0[a]
Acetoacetate[f]	I	1.6 ± 0.5	1.0 ± 0.1	0.3 ± 0.2[a]	−0.9 ± 0.5[a]	−0.9 ± 0.4[a]
	II	1.5 ± 0.9	1.9 ± 0.5[a]	2.5 ± 0.7[a,b]	2.9 ± 0.7[a,b]	3.3 ± 1.0[a,b]
	III	1.2 ± 0.4	0.6 ± 0.2[c]	0.4 ± 0.3[a,c]	0.4 ± 0.3[a,c]	0.6 ± 0.4[a,c]

The values represent the mean ± SEM of 4 healthy subjects in ml per 100 g of muscle weight per min[d], in ml per 100 ml[e] and in μmoles per 100 ml[f]. [a] Indicates significant difference to basal (paired t-test, $p < 0.05$), [b] as compared to I (unpaired t-test, $p < 0.05$) and [c] as compared to II (unpaired t-test, $p < 0.05$) Further details under Materials and methods.

exclusively via the acceleration of blood flow was evident from the simultaneous reestablishment of muscular glucose extraction (table 1). Unspecific increase of the membranes permeability, as a cause of enhanced glucose uptake, was not very probable since simultaneous uptake of the residual substrates as, i.e., that of ketones was reduced (table 1).

That the enhanced glucose uptake during exercise represented only greater influx of glucose into the inter- or intracellular space, was also not very likely, since the relatively large amounts of glucose did not leave the muscular tissue again when the work was discontinued (table 1).

A physiological role of the kallikrein–kinin system in muscle may be deduced also from earlier findings. Thus, lowering of blood pH leads to enhanced kallikrein formation from prekallikrein [12] and to a reduction of the activity of kininases [13]. Furthermore, muscular exercise was found to depress the plasma concentration of kininogen and to increase that of kinins [14].

Whether the action of bradykinin on muscular glucose uptake described here is independent of insulin, or whether the kallikrein–kinin-system is involved in insulin action, needs further investigation. It appears of interest, however, that bradykinin applied into the brachial artery during muscular rest also accelerated muscular glucose entry at the cell surface [15].

Moreover, it has been observed that small insulin concentrations are necessary for the increase of muscular glucose transport during exercise [3] and that insulin may reduce the activity of kininases [16].

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