INSULIN ACTION ON GLUCOSE UPTAKE INTO SKELETAL MUSCLE

Inhibition of endogenous biosynthesis of prostaglandins

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1. Introduction

We demonstrated [1] that insulin action on glucose uptake into skeletal muscle is significantly reduced when the proteolytic liberation of kinins from kininogen by kallikrein was suppressed by a kallikrein inhibitor. A crucial role of kinin liberation has been demonstrated for the acceleration of glucose uptake which occurs during muscle work [2] and hypoxia [3]. Since evidence had been obtained [4] that the effect of kinins on glucose uptake into muscle was mediated via the synthesis of prostaglandins, it was of interest to investigate whether insulin action on glucose uptake into skeletal muscle of the human forearm might be impaired after indomethacin pretreatment which prevents prostaglandin synthesis in man [5].

2. Materials and methods

Fourteen healthy volunteers were recruited from medical students. All were informed about the aim and the risks of the study and gave their consent. Physical examination as well as laboratory tests excluded internal diseases. All the subjects fasted overnight and received no special premedication. The catheterization procedure was detailed in [3,4]. Arterial and deep-venous blood samples were collected simultaneously at 5 min intervals throughout 15 min basal period followed by a 30 min infusion period for chemical analysis.

Chemical analysis was performed in 6 subjects (group 1) during the intrabrachial–arterial infusion of highly purified crystalline bovine insulin (250 μU/kg × min in 0.2 ml physiological saline/min) for the whole test period. Another 6 subjects (group 2) received identical insulin infusion after the oral pretreatment with indomethacin Amuno® from Sharp and Dohme, Munich (3 daily doses of 100 mg each for 2 days and another 100 mg dose 1 h prior to the test). The 2 groups were well comparable as to their age, height and weight. Three minutes after the start of insulin infusion, 1 mg glucose/kg body wt/min was infused into an anticubital vein in group 1. Forearm blood flow was estimated by venous-occlusion plethysmography [6,7] as detailed in [4].

Glucose, free fatty acids and β-hydroxybutyrate were determined after storage at -20°C overnight, acetoacetate at least within 6 h. Blood samples for gas analysis were taken in heparinized syringes and analyzed promptly. Procedure and precision of the tests has been given in [8]. Serum insulin was assayed according to a modification [9] of the Yalow-Berson immunoassay [10], human growth hormone (HGH) according to [11]. Standard statistical methods were employed using Student's t-test for paired and unpaired samples when applicable [12]. All the mean values are given with the standard error of the mean (SEM).

3. Results

The arterial concentration of substrates and of HGH are listed in table 1. After indomethacin pretreatment glucose concentration was significantly

Table 1
Arterial concentrations of substrates and of human growth hormone (HGH) during the intrabrachial-arterial infusions of insulin (I) and insulin after indomethacin pretreatment

			Insulin[e]		
		Basal[d]	10 min	20 min	30 min
Oxygen[a]	I	19.9 ± 0.3	19.9 ± 0.3	–	20.0 ± 0.3
	II	19.6 ± 0.2	19.7 ± 0.2	–	19.8 ± 0.2
Glucose[b]	I	4.52 ± 0.10	4.60 ± 0.11	4.53 ± 0.11	4.42 ± 0.10
	II	5.25 ± 0.18[g]	5.40 ± 0.16[g]	5.28 ± 0.18[g]	5.28 ± 0.18[g]
Free fatty acids[b]	I	0.743 ± 0.098	0.687 ± 0.065	0.620 ± 0.081	0.610 ± 0.086[f]
	II	0.848 ± 0.101	0.798 ± 0.877	0.720 ± 0.061	0.617 ± 0.059[f]
β-Hydroxybutyrate[b]	I	0.125 ± 0.018	0.125 ± 0.018	0.120 ± 0.020	0.115 ± 0.018
	II	0.168 ± 0.030	0.165 ± 0.030	0.153 ± 0.028	0.148 ± 0.024
Acetoacetate[b]	I	0.074 ± 0.012	0.075 ± 0.017	0.075 ± 0.016	0.068 ± 0.016
	II	0.071 ± 0.020	0.065 ± 0.017	0.061 ± 0.015	0.056 ± 0.014
HGH[c]	I	7.5 ± 4.1	–	–	–
	II	9.6 ± 4.2	–	–	–

The values are given as the mean ± SEM of 6 (I) and 8 (II) subjects in ml/100 ml[a] and in mmol/l[b], the values for HGH as the mean ± SEM of 6 subjects in each group in ng/ml[c], [d] from 4 determinations at 5 min intervals averaged for each subject, [e]250 μU kg body $wt^{-1} \cdot min^{-1}$. [f]Significant at $P < 0.05$ to basal, [g]at $P < 0.05$ to I.

higher and did not fall during the infusion of insulin although no glucose was infused. The arterial concentrations of growth hormone were almost identical in both collectives.

Forearm blood flow (FBF), arterial–deep-venous concentration difference of oxygen and glucose, and deep-venous concentration of insulin (IRI) are given in table 2.

Arterial–deep-venous glucose difference rose continuously reaching 5-times the basal value at the end of insulin infusion ($p<0.005$, paired t-test). After indomethacin pretreatment this effect of insulin was significantly reduced throughout the test (table 2). Deep-venous IRI concentrations exhibited no significant differences between both groups. While the calculated uptake of oxygen was maintained, glucose uptake rose corresponding to the increase of arterial–deep-venous glucose difference as illustrated in fig.1. After indomethacin treatment glucose uptake was significantly reduced while oxygen uptake remained essentially unchanged.

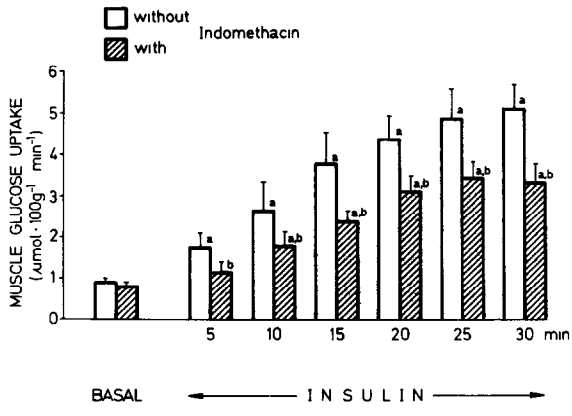

Fig.1. Glucose uptake into skeletal muscle of the forearm. The values were calculated from the arterial deep-venous differences and the corresponding blood flow rates and are indicated as the mean ± SEM of 6 subjects in each group. The values for the controls have been obtained from [1]. Insulin was infused into the brachial artery. (a) Indicates significant difference at $p<0.05$ as compared to basal; (b) at $p<0.05$ as compared to untreated subjects.

Table 2

Forearm blood flow (FBF), arterial–deep-venous concentration differences of oxygen and glucose and deep-venous concentrations of insulin (IRI) during the intrabrachial–arterial infusions of insulin (I) and insulin after indomethacin pretreatment (II)

			Insulin[f]					
		Basal[e]	5 min	10 min	15 min	20 min	25 min	30 min
FBF[a]	I	2.67 ± 0.43	2.87 ± 0.17	2.60 ± 0.21	2.67 ± 0.31	2.80 ± 0.30	2.90 ± 0.41	2.87 ± 0.32
	II	2.75 ± 0.32	2.75 ± 0.30	2.75 ± 0.29	2.80 ± 0.30	2.80 ± 0.27	2.88 ± 0.35	2.80 ± 0.31
Oxygen[b]	I	9.6 ± 0.2	9.8 ± 0.6	10.1 ± 1.0	10.1 ± 1.5	10.5 ± 1.5	10.5 ± 1.4	9.8 ± 1.1
	II	9.2 ± 0.7	9.2 ± 0.8	9.1 ± 0.9	8.8 ± 1.0	8.5 ± 1.0	8.5 ± 1.0	8.5 ± 1.1
Glucose[c]	I	39.0 ± 3.3	72.0 ± 9.8[g]	114.0 ± 19.3[g]	161.5 ± 22.4[g]	184.3 ± 13.9[g]	196.2 ± 10.2[g]	199.3 ± 5.6[g]
	II	35.0 ± 4.6	55.6 ± 8.9[g,h]	78.1 ± 11.2[g,h]	93.8 ± 15.1[g,h]	119.4 ± 15.9[g,h]	125.6 ± 16.6[g,h]	120.6 ± 18.2[g,h]
IRI[d]	I	8.3 ± 1.1	–	168.4 ± 19.6[g]	–	156.0 ± 24.9[g]	–	188.0 ± 25.2[g]
	II	9.8 ± 1.5	–	135.2 ± 20.1[g]	–	130.4 ± 21.4[g]	–	151.2 ± 20.3[g]

The values for oxygen and glucose are given as the mean ± SEM of 6 (I) and 8 (II) subjects in ml/100 ml[b] and μmol/100 ml[c]. The values for FBF and IRI as the mean ± SEM of 6 subjects in each group in ml·100 g tissue^{-1}·min^{-1}[a] and μU/ml[d,e] From 4 determinations at 5 min intervals averaged for each subjects.[f] 250 μU·kg body wt^{-1}·min^{-1}.[g] Significant at $P<0.05$ to basal.[h] Significant at $P<0.01$ to I

4. Discussion

Insulin accelerated glucose uptake into skeletal muscle of the human forearm as described [13,14]. The well-known insulin resistance occurring after indomethacin pretreatment [15–17] (manifested by elevated glucose concentrations in the presence of normal insulin levels and the reduced action of insulin on glucose uptake into muscle) could not be attributed to an increase in growth hormone (table 1,2). The effect of indomethacin during insulin infusion could also not be ascribed to a smaller insulin supply (table 2). Furthermore, the data on oxygen uptake, free fatty acids and ketone bodies indicated comparable metabolic conditions in both groups and thus, provided likewise no explanation for the reduced glucose utilization after indomethacin.

It appears therefore, that the inhibition of prostaglandin biosynthesis by indomethacin is responsible for the depression of insulin activity [5]. This would imply a participation of prostaglandins in the translation of insulin action on glucose uptake into skeletal muscle. There are other findings which favour this view. Prostaglandins are known to exhibit insulin-like activity in myocardial [18–20] and adipose tissue [21,22].

Furthermore, kinins, the most likely candidates for the muscular activity factor [2,3,23] (which themselves display insulin-like activity [4,24]) are no longer effective if prostaglandin synthesis is blocked by indomethacin [4]. Further support has come from the finding that prevention of endogenous kinin liberation by a kallikrein inhibitor also reduced the action of insulin on glucose uptake into the human forearm [1]. That insulin, in contrast to kinins [4,25] and prostaglandins [26], did not accelerate blood flow ([13,14] table 2) would not speak against such a notion, since insulin exhibits its actions only in insulin-sensitive tissues where the hormone is bound to specific receptors [27].

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