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Rapid communication

Effect of human C-peptide on glucose transport in in vitro incubated human skeletal muscle

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Summary. Muscle specimens from the quadriceps femoris muscle were obtained from eight healthy subjects by means of an open muscle biopsy and prepared for in vitro incubation. C-peptide at 0.5, 1.0 and 2.5 nmol/l increased 3-0-methylglucose transport by 38% (NS), 64% ($p < 0.05$), and 64% ($p < 0.05$) respectively. Glucose transport increased 1.8-fold in the presence of 0.3 nmol/l of insulin ($p < 0.05$). Glycogen content in muscle strips exposed to C-peptide at a concentration of 1 nmol/l increased significantly by 22% ($p < 0.05$). In conclusion, C-peptide stimulates the rate of 3-0-methylglucose transport in in vitro incubated human skeletal muscle strips in a dose-response manner. These observations suggest that C-peptide may contribute to the regulation of carbohydrate metabolism in human skeletal muscle.

Key words: C-peptide, glucose transport, human, insulin, skeletal muscle.

C-peptide is a 31-amino acid peptide that connects the alpha and beta chains of the insulin molecule [1], and is released from the pancreatic Beta cells in concentrations equimolar to insulin [2]. In alloxan-diabetic rats, a supraphysiological concentration of C-peptide increased and prolonged the hypoglycaemic effect of exogenous insulin [3]. However, physiological levels of C-peptide have been reported to have no biological effect upon glucose metabolism in peripheral tissues [4–6].

We have utilized an open muscle biopsy technique to obtain muscle specimens from the vastus lateralis portion of the quadriceps femoris muscle. Using this technique, we investigated the effects of increasing concentrations of C-peptide or insulin on the rate of 3-0-methylglucose transport in incubated human muscle strips.

Subjects and methods

Subjects

Muscle specimens were obtained from the vastus lateralis portion of the quadriceps femoris muscle from eight healthy male subjects (age 28.5 ± 1.8 years, BMI 23.7 ± 0.8 kg/m^2). None of the subjects were taking any medication. The nature, purpose, and possible risks of the study were reviewed and approved by the Ethical Committee of the Karolinska Institute, and were carefully explained to each individual before they gave consent to participate. Following an overnight fast, the subjects reported to the laboratory at 08.30 hours. A local anaesthesia (prilocain hydrochloride 10 mg/ml) was administered subcutaneously 15 cm above the proximal border of the patella and a 4 cm incision was made. Two muscle specimens (250 mg each) for in vitro incubation were excised from the muscle and smaller strips were prepared as described earlier [7, 8].

Incubation procedure and 3-0-methylglucose transport measurements

After preparation, the smaller muscle strips (approximately 20 mg) were incubated at 35 °C for 10 min in a solution containing oxygenated Krebs-Henseleit's bicarbonate buffer supplemented with HEPES (N-2-Hydroxyethyl-piperazine-N′-2-ethanesulphonic acid) (KHB), 38 mmol/l mannitol, 2 mmol/l pyruvate and 0.1% bovine serum albumin (BSA). The strips were subsequently pre-incubated for 2 h in KHB containing 35 mmol/l mannitol, 5 mmol/l glucose and the concentration of insulin or C-peptide as stated in Figure 1 and Table 1. Immediately after pre-incubation the muscle strips were incubated for 20 min in KHB medium, in which 5 mmol/l 3-0-[^{3}H]methylglucose (437 μCi/mmol) and 35 mmol/l [^{14}C]mannitol (8 μCi/mmol) were substituted for unlabelled glucose and mannitol respectively. Following incubation, the muscles were processed as described earlier [9].

Biochemical assays

Portions of the incubated muscle strips were rapidly freeze-clamped with aluminium tongs cooled to the temperature of liquid nitrogen and analysed for glycogen content as previously described [9]. To

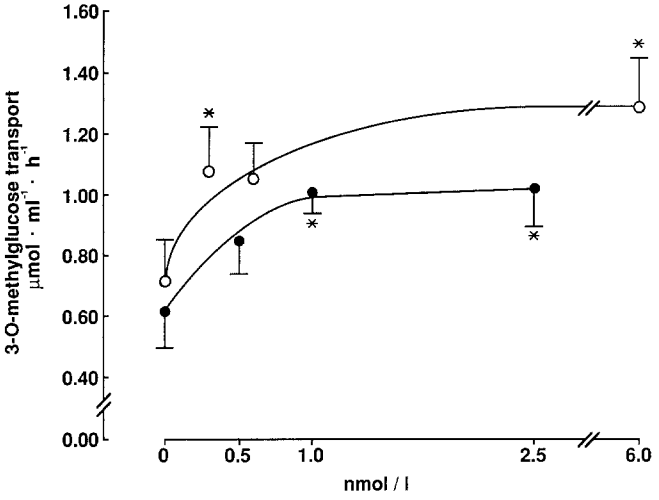

Fig. 1. Dose-response curve for insulin- (○) or C-peptide- (●) stimulated human skeletal muscle strips. *$p < 0.05$ vs non-stimulated skeletal muscle strips. Values are expressed per ml of intracellular water. Values are means ± SEM for 5–7 muscles

determine if the C-peptide was contaminated with insulin, 170 µmol/l of C-peptide was assayed for insulin by the Phadeseph insulin RIA method (Pharmacia, Uppsala, Sweden) and found to be below the detection limit of the assay.

Chemicals

All chemicals were obtained from Sigma Chemical Company (St. Louis, Mo., USA) and the radioactive products were purchased from New England Nuclear (Boston, Mass., USA). The insulin (Actrapid) was a product of Novo Nordisk A/S (Copenhagen, Denmark) and the human C-peptide was purchased from Bachem Feinchemikalien AG (Bubendorf, Switzerland; 98.8% purity, HPLC). Conversion factor for insulin is 1 nmol/l = 167 µU/ml.

Statistical analysis

The statistical significance of the differences between the glycogen levels in the muscle strips incubated in the presence or absence of C-peptide or insulin was determined by Student's paired *t*-test. A one-way analysis of variance (ANOVA) was employed to evaluate the differences between various concentrations of C-peptide or insulin. When the ANOVA resulted in a significant F-value, the difference between the means was identified by the Newman-Keul test. A significance level of $p < 0.05$ was used. Results are expressed as means ± SEM.

Table 1. Effect of C-peptide and insulin on skeletal muscle glycogen content

Perturbation	*n*	Glycogen (mmol · kg dry weight^{-1})		
		Basal	Perturbation	Change
C-peptide 1 nmol/l	6	282 ± 28	343 ± 22	+ 22 %[a]
Insulin 0.6 nmol/l	5	292 ± 32	373 ± 47	+ 28 %

Values are expressed per ml of intracellular water. Values are means ± SEM.
[a] $p < 0.05$ vs basal

Results

C-peptide increased the rate of 3-0-methylglucose transport in a dose-response manner (Fig. 1). At the lowest concentration, 0.5 nmol/l, the 3-0-methylglucose transport was not significantly different from the basal transport rate (0.62 ± 0.12 vs 0.85 ± 0.11 µmol · ml^{-1} · h^{-1}, NS). At concentrations of 1.0 and 2.5 nmol/l, C-peptide significantly increased the rate of basal glucose transport by 64% from 0.62 ± 0.12 to 1.02 ± 0.08 ($p < 0.05$) and 1.02 ± 0.12 ($p < 0.05$) µmol · ml^{-1} · h^{-1}, respectively (Fig. 1). Glucose transport increased 1.8-fold, with a maximal effect obtained in the presence of 0.3 nmol/l of insulin (Fig. 1). After a 2-h in vitro incubation, in the presence of 5 mmol/l glucose, C-peptide increased the muscle glycogen levels by 22 % ($p < 0.05$, Table 1). In the insulin-stimulated muscles, glycogen content was not altered (NS).

Discussion

With the open muscle biopsy technique, it is now possible to obtain intact human skeletal muscle which can be used for in vitro metabolic studies [7, 8]. In the present report, the maximal stimulatory effect of insulin on glucose transport was observed at 0.3 nmol/l. Since serum insulin concentrations in humans are generally observed in a range of 0.05–0.2 nmol/l, the in vitro system employed in the present investigation is sensitive to physiological concentrations of insulin. The amino acid sequence of C-peptide is species-specific [10]; thus it is necessary to use human C-peptide to investigate the biological effects of this peptide in human tissue.

Here we demonstrate that physiological concentrations of C-peptide stimulate the basal rate of glucose transport in human skeletal muscle in a dose-response manner with V_{max} at a concentration of 1.0 nmol/l C-peptide. The increase in skeletal muscle glycogen content following a maximal C-peptide stimulation was similar in magnitude when compared to the effect of 0.6 nmol/l of insulin. However, due to a limited number of muscle samples, and the large variability in the insulin effect on glycogen synthesis, significance was not obtained for the insulin-stimulated muscles. In conclusion, the increase in glucose transport and glycogen content of in vitro incubated human skeletal muscle provides evidence for a regulatory role of C-peptide in carbohydrate metabolism. Additional studies using human C-peptide and human skeletal muscle are imperative to assess the mechanism governing this reported biological activity for C-peptide in human skeletal muscle.

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Announcements

27th Annual Meeting of the European Diabetes Epidemiology Study Group of the European Association for the Study of Diabetes

This meeting will be held in Bremen, FRG, from 24–27 May 1992. *Deadline for the submission of abstracts:* 1 April 1992. *For further information please contact:* Prof. Dr. H. U. Janka, Local Organiser, Zentralkrankenhaus Bremen-Nord, Hammersbecker Strasse 228, D-2820 Bremen, FRG. *Secretary of the EDESG:* Dr. K. Borch-Johnsen, Steno Memorial Hospital, Niels Steensensvej 2, DK-2820 Gentofte, Denmark, Fax: (45) 31 68 23 22.

16th World Congress of the International Union of Angiology

This congress will be held from 13–18 September 1992, in the Palais des Congrès, Paris, France. *Deadline for submission of abstracts:* 31 December 1991. *Official language:* English. *For further information please contact:* Prof. H. Boccalon, CHU Rangueil, Service d'Angiologie, 1, Avenue J Poulhès, F-31054 Toulouse Cédex, France, Tel: (33) 61 32 29 45, Fax: (33) 61 32 26 34. *Administrative Secretariat:* S.O.C.F.I., Angiology Congress, 14, Rue Mandar, F-75002 Paris, France. Tel: (33) 42 33 89 94, Fax: (33) 40 26 04 44.