Biochimica et Biophysica Acta, 389 (1975) 194—196

BBA Report

BBA 71223

INSULIN AND INSULIN INHIBITOR ON AMINO ACID TRANSPORT IN RAT DIAPHRAGM IN VIVO

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(Received February 12th, 1975)

Summary

Insulin stimulated the accumulation of α-aminoisobutyric acid by the rat diaphragm in vivo. This stimulation, but not the accumulation in the absence of insulin, was reduced by 30—40% by insulin inhibitor present in extracts of liver.

The hyperinsulinaemia seen in obesity and in obese, maturity-onset diabetes is generally ascribed to peripheral resistance to insulin [1, 2]. The nature of this resistance is not known, but one possibility is the existence of a humoral factor that inhibits the action of insulin on muscle. Insulin inhibitory activity of this kind has in fact been demonstrated in the sera of several mammalian species [3, 4], and an activity with identical properties has been found in extracts of liver [5, 6]. This activity is due to polypeptide(s) of M_r = 5000—10 000 [4, 6], and for conciseness it will be referred to as 'inhibitor'. The activity was shown by using the intraperitoneal test of Rafaelsen et al. [10], where the effect of injected insulin can be measured on both the diaphragm and the epididymal adipose tissue. Since in those experiments the inhibitor was without effect on insulin's action on adipose tissue, whereas in the same animals it reduced insulin's action on diaphragm [3, 6], the inhibitor cannot be combining with insulin to give an inactive complex. This is confirmed by the dose-response curve for the inhibitor being a linear relation between degree of inhibition of insulin action on the diaphragm and log concentration of inhibitor [6].

To determine whether the inhibitor acts on the reaction of insulin with the membrane receptors rather than on a reaction specifically linked to glucose and its subsequent metabolism, another substance (α-aminoisobutyric

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acid) was chosen of which the uptake by the diaphragm, at least in vitro, is stimulated by insulin [7—9] and, furthermore, that is not metabolized. Using the intraperitoneal test, we found that insulin stimulated the uptake of α-aminoisobutyric acid by diaphragm, and that this stimulation was inhibited by extracts of liver to the same degree. Therefore the inhibitor acts on the insulin stimulation of carrier systems of transport and presumably on the binding of insulin to membrane receptors.

The experimental procedures for the intraperitoneal method have been described in detail elsewhere [3, 11]. Into fasted (24 h), male Wistar rats weighing 80—100 g 1.5 ml of a solution was injected containing bovine serum albumin (3 g/100 ml), NaCl (154 mM), Evans Blue conjugate (2.5 mg dye plus 61 mg albumin per 100 ml), α-amino[1-^{14}C]isobutyric acid ($6.5 \cdot 10^{-6}$ M, spec. act. 10 Ci/mol; Radiochemical Centre, Amersham, England) and, when used, insulin (1 munit per rat) and liver extracts (4—8 mg per rat).

Liver extracts were prepared by boiling liver homogenates in saline (0.9 g/100 ml) at pH 4.5 for 15 min. The cooled suspension was filtered, diluted to 4 times its volume with water, and glacial acetic acid added to give a final concentration of 0.1 M acetic acid. The total volume was passed through a column of IRC-50 ion-exchange resin (H^+) equilibrated with 0.1 M acetic acid. After the column had been washed with 0.1 M acetic acid, the adsorbed material was eluted with glacial acetic acid. Evaporation and freeze-drying of this solution yielded the solid preparation (3.5 g from 1 kg pig liver).

The rats were killed 60 or 120 min after the intraperitoneal injection. The diaphragm and segments of the rectus femoris muscle were rapidly excised, rinsed in ice-cold saline (9 g/l) for 10 s and frozen in acetone containing solid CO_2. The muscle was homogenized in 2 ml ice-cold 5% trichloroacetic acid. After centrifugation of the homogenates, 1 ml aliquots of the supernatant fluid were added to 10 ml samples of dioxane scintillation medium [12] and counted in a liquid scintillation spectrometer. Blood samples were collected when the animals were decapitated, and 0.1 ml of the serum was mixed with 1 ml 5% trichloroacetic acid and 10 ml of the same scintillation medium. By internal standardization, the counting efficiency was found to be the same for muscle and serum extracts, thus allowing their direct comparison.

Fig. 1 shows that after intraperitoneal injection of α-amino[1-^{14}C]iso-

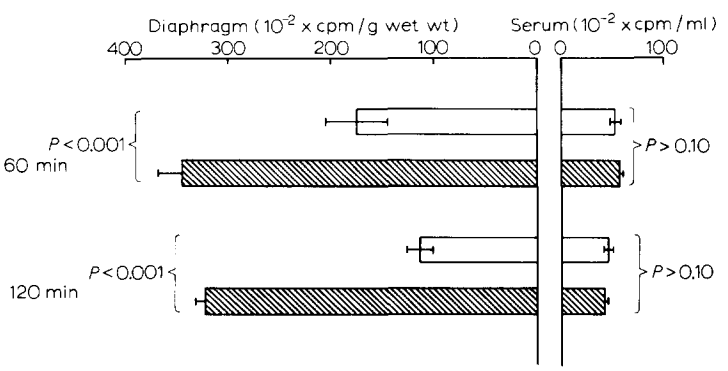

Fig. 1. Effect of insulin in vivo (1 munit/rat) (hatched bars) on the accumulation of α-amino[1-^{14}C]isobutyric acid in the rat diaphragm and on the level of the amino acid in the serum. Rats were killed 60 or 120 min after intraperitoneal injection of a mixture of insulin and the amino acid.

butyric acid, the levels of radioactivity in serum and diaphragm muscle indicate an accumulation of this amino acid in the muscle cells. When insulin was added to the solution injected, there was a 2—3-fold increase in the accumulation of α-aminoisobutyric acid in the diaphragm, whereas the ^{14}C activity of the serum was unchanged. Urine samples, which were collected just before the animals were killed, showed that insignificant amounts of radioactivity had been excreted within the experimental period. Rectus femoris muscle also took up α-amino[1-^{14}C]isobutyric acid, but there was no difference between the activity recovered from the controls and that from insulin-treated animals. These findings show that intraperitoneally injected insulin stimulated the accumulation of α-aminoisobutyric acid in diaphragm muscle without producing peripheral effects, a response paralleling that already described for glycogen synthesis [10]. Unlabelled α-aminoisobutyric acid added to the solution for injection ($1.3 \cdot 10^{-4}$ M), reduced the accumulation of ^{14}C activity in the diaphragm, and the effect of insulin was also diminished. This agrees with the finding for intact diaphragm in vitro [13]. A low concentration of α-aminoisobutyric acid and an experimental period of 2 h gave the optimal insulin response and minimal statistical variation (Fig. 1).

The effects of partially purified extracts of liver containing insulin inhibitor were assessed in the presence and absence of insulin (Table I). Whereas

TABLE I

EFFECT OF INSULIN (1 munit/rat) AND OF THE INSULIN INHIBITOR IN EXTRACTS OF PIG AND RAT LIVER ON THE ACCUMULATION OF α-AMINO[1-^{14}C]ISOBUTYRIC ACID IN RAT DIAPHRAGM IN VIVO

Results are given as 10^{-2} x cpm/g wet wt of diaphragm with S.E. Each group consisted of seven rats. Liver extracts: Expts I and II, two separate preparations of pig liver extract (8.5 mg per rat, corresponding to 2.4 g fresh liver; Expt III, rat liver extract (4.3 mg per rat, corresponding to 2 g of fresh liver).

	Expt I	*P*	Expt II	*P*	Expt III	*P*
α-Aminoisobutyric acid	117 ± 10	> 0.10	117 ± 9	> 0.10	120 ± 19	> 0.10
α-Aminoisobutyric acid + liver extract	134 ± 10		121 ± 12		115 ± 21	
α-Aminoisobutyric acid + insulin	282 ± 22	< 0.02	324 ± 29	< 0.001	301 ± 19	< 0.001
α-Aminoisobutyric acid + insulin + liver extract	195 ± 20		188 ± 6		185 ± 17	

the liver extract did not affect the accumulation of α-aminoisobutyric acid in the absence of insulin, its administration along with insulin reduced by 30—40% the enhanced accumulation of α-aminoisobutyric acid caused by that hormone. When the activities of the same liver extracts were determined in experiments using the incorporation of [^{14}C]glucose into diaphragm glycogen as the index of insulin activity, a 30—35% reduction of the effect of 1 munit of insulin was found.

In agreement with an earlier report [14], α-aminoisobutyric acid accumulates in the rat diaphragm in vivo; and the results show that the intraperitoneal method can be used to demonstrate a reproducible stimulation by insulin of α-aminoisobutyric acid transport into diaphragm. Riggs and McKirahan [9] have suggested that there are three saturable routes for entry of amino acids into rat diaphragm, and that α-aminoisobutyric acid is transported almost totally by one system, which is the one sensitive to insulin. Liver extracts that reduced the effect of insulin on glycogen synthesis, significantly and reproducibly inhibited to the same extent the insulin stimulation

of α-aminoisobutyric acid accumulation in the diaphragm. Since these extracts were without effect on the accumulation of α-aminoisobutyric acid in the absence of insulin, the conclusion must be that the inhibitor acts on the insulin stimulation of the carrier systems for α-aminoisobutyric acid and glucose, probably at the insulin receptor, and not on the systems themselves nor on the disposition of these substances subsequent to transport.

This work was supported by the Fonds National Suisse de la Recherche Scientifique, Grant No. 4848.3, and a Grant-in-Aid, Sandoz Pharmaceutical Co. Ltd, Basel, Switzerland.

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