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Insulin receptor binding and tyrosine kinase activity in liver and skeletal muscle from fasted rats

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Summary — Insulin binding and tyrosine kinase activity of the insulin receptor have been measured in the liver and muscles of rats fed or submitted to a 72-h-fasting. In both tissues, insulin binding increased in fasting rats. In liver, the ability of insulin to simulate receptor tyrosine kinase activity greatly unpaired during fasting, but remained unchanged in muscle. The change during fasting of the insulin-stimulated tyrosine kinase activity of the insulin receptor is specific to certain tissue.

insulin — receptor — tyrosine kinase activity — rat — fasting

Résumé — Récepteurs d'insuline et activité tyrosine kinase dans le foie et le muscle du rat en croissance : influence d'un jeûne de 72 h. *La fixation d'insuline et l'activité tyrosine kinase du récepteur ont été mesurées dans le foie et le muscle squelettique (préparations de membranes microsomiales solubilisées et purifiées par chromatographie d'affinité) du rat nourri ou soumis à un jeûne de 72 h. Dans les deux tissus, la fixation d'insuline est augmentée chez les rats soumis au jeûne. Dans le foie, la capacité de l'insuline à stimuler l'activité tyrosine kinase du récepteur est fortement diminuée au cours du jeûne alors qu'elle est inchangée dans le muscle. La variation de réponse à l'insuline de l'activité tyrosine kinase du récepteur induite par le jeûne présente donc une spécificité tissulaire.*

insuline — récepteur — tyrosine-kinase — rat — jeûne

INTRODUCTION

Fasting has been shown to alter insulin action *in vivo, i.e.,* there was an impairment in insulin responsiveness in both glucose utilization and production in rats (Penicaud *et al.,* 1985). *In vitro,* insulin responsiveness was also impaired in liver (Cech *et al.,* 1980), but not in muscle (Brady *et al.,* 1981). Thus, in order to clarify the role of insulin receptor interaction in the regulation of insulin action during starvation, we measured insulin binding and insulin receptor tyrosine kinase activity in both liver and skeletal muscle of 72-h-fasted rats.

ANIMALS AND TREATMENTS

Male Wistar rats (150 g), housed in controlled environmental conditions (22 °C, 60% relative humidity and 12 h dark period) were either fed *ad libitum* a standard rat chow or fasted for 72 h. Livers and skeletal muscles from hind legs were removed, and kept at – 80 °C until use. Insulin receptors (microsomal membranes) were then prepared, according to standard methods of Havrankova, Roth and Browstein (1978) and Hedo *et al.* (1981). Insulin binding and insulin receptor tyrosine kinase activity (assessed by the ability of insulin to stimulate the phosphorylation of the artificial exogenous substrate poly Glu-Tyr 4:1) were measured in partially wheat germ agglutinin-purified insulin receptors from both tissues.

RESULTS AND DISCUSSION

Insulin binding to liver and skeletal muscle receptor preparations was approximately 2 fold higher in fasted rats compared with fed rats. According to Scatchard's analysis, the enhanced insulin binding in both liver and skeletal muscle of fasted rats appeared to be due to an increase in the number of insulin binding sites (liver : 89 ± 7 *vs* 46 ± 2 pmols/mg protein; muscle : 61 ± 7 *vs* 36 ± 6 in fasted and fed group, respectively; mean ± SEM) without any change in binding affinity. Improvement of insulin binding to both tissues from fasted rats appeared to agree with the increased insulin binding previously described in liver plasma membranes, hepatocytes, or isolated soleus of fasted rodents (Almira & Reddy, 1979; Herrera *et al.,* 1981; Brady *et al.,* 1981; Le Marchand-Brustel & Freychet, 1979). These increases correlated with the decrease in plasma insulin generally observed in fasted rats (Penicaud *et al.,* 1985).

In liver, insulin-stimulated tyrosine kinase activity decreased significantly in fasted rats when compared to fed rats, whereas, basal activity was unchanged (Table I). Thus, the ability of insulin to stimulate the insulin receptor tyrosine kinase was greatly depressed during starvation. Contrary to liver activity, both basal and insulin stimulated kinase activity of muscle insulin receptors were unchanged in fasted and fed rats (Table I). Insulin sensitivity (determined as the insulin concentration required for 50% maximal stimulation) was not affected by nutritional state in both tissues.

The increased insulin binding sites, in both liver and skeletal muscle, could explain the apparent improved insulin sensitivity on hepatic glucose production or peripheral glucose utilization observed *in vivo* in fasted rats (Penicaud *et al.,* 1985). In addition, our results confirmed that fasting altered the insulin receptor kinase activity in liver (Freidenberg *et al.,* 1985; Simon *et al.,* 1986). Such modification was consist-

Table I. Insulin receptor tyrosine kinase activity in liver and skeletal muscle from fasted and fed rats.

	Liver		*Muscle*	
	Fed	*Fasted*	*Fed*	*Fasted*
Basal activity	2.7 ± 0.4	1.6 ± 0.3	20 ± 4	25 ± 6
Maximal insulin	33.6 ± 5.7	14.8 ± 2.8*	113 ± 19	102 ± 13

Values are means ± SE of five individual experiments. They are expressed as fmoles ^{32}P incorporated/mg substrate/min/pmole binding capacity.* $P < 0.05$.

ent with the insulin unresponsiveness observed *in vivo* (Penicaud *et al.*, 1985) or *in vitro* (Cech *et al.*, 1980) in fasted rats. In skeletal muscle, normal insulin receptor tyrosine kinase during starvation supports the concept that the same metabolic change did not necessarily induce the same alterations of insulin receptor tyrosine kinase activity in different tissues.

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