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IN VIVO EVIDENCE THAT INSULIN DOES NOT INHIBIT HEPATIC TRYPTOPHAN PYRROLASE ACTIVITY IN RATS

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Abstract—Previous reports have indicated that insulin administration triggers an early increase in plasma tryptophan (TRP) levels in fasted rats. Then, the present study was undertaken to investigate the putative role of liver tryptophan pyrrolase (TPO) in this short-term effect of insulin. In 24 hr fasted rats, doses of insulin that triggered an increase in plasma TRP levels (i.e., 2–3 I.U./kg, 1 hr) did not alter either holoenzyme or total enzyme activity. In another series of experiments, the administration of insulin (2 I.U./kg) to 24 hr fasted rats promoted biphasic time effects on plasma TRP levels and liver TPO activity. Thus, insulin initially triggered a rise in plasma TRP (without any change in liver TPO activity) and then increased liver TPO activity whilst plasma TRP returned toward control levels. In addition, hypercorticosteronemia was evidenced throughout the first phasis. Lastly, the influence of insulin administration (2 I.U./kg) on fasting-induced TPO induction was analysed. Whereas fasting increased liver TPO activity in a time-dependent manner, insulin administration (2 I.U./kg, 30 min) did not modify either plasma TRP or liver TPO activity. The data reported herein bring evidence that the effect of insulin administration on circulating TRP is not mediated by an inhibition of hepatic TPO.

Brain serotonin (5-hydroxytryptamine, 5HT) synthesis may vary following changes in the concentration of brain tryptophan (TRP), its precursor, inasmuch as the rate-limiting enzyme for 5HT synthesis, namely tryptophan hydroxylase, is unsaturated with regard to its substrate TRP [1, 2]. In fasted rats, insulin administered exogenously (or secreted in response to carbohydrate feeding) elevates brain TRP and 5-hydroxyindole levels because hyperinsulinemia increases plasma TRP concentration and lowers the circulating level of a group of large neutral amino acids (LNAA) that compete with TRP for uptake into the brain [3–6]. Whereas insulin-induced acceleration of amino acid uptake into peripheral tissues can contribute to the decrease of LNAA plasma levels, the mechanism(s) by which hyperinsulinemia increases plasma TRP level is (are) at the present time still unknown.

One of the determinants of TRP availability to various tissues (including the brain) is the activity of liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11) (TPO) [7]. Thus, this enzyme catalyses the first and rate limiting step of the liver kynurenine pathway, the most important route of TRP metabolism in rats. Consequently, changes in TPO activity can influence plasma TRP concentration and then brain TRP availability [8–10].

Taking into consideration the above related data, it could be that changes in liver TPO activity are (partly or totally) responsible for plasma TRP elevation in insulin-treated rats. Such an hypothesis is reinforced by the finding that brain TRP level is decreased as a result of increased liver TPO activity in streptozotocin-diabetic rats [11–13]. Moreover, the induction of TPO by glucagon and dexamethasone has been reported to be dose-dependently inhibited by insulin in primary cultured rat hepatocytes [14], thus suggesting again a role for insulin as a regulator of liver TPO.

Accordingly the hypothesis that hyperinsulinemia increases plasma TRP level by means of an inhibition of hepatic TPO was tested herein. Inasmuch as fasting activates liver TPO activity [15], the *in vivo* effects of insulin administration on hepatic TPO activity, plasma TRP and corticosterone levels were evaluated in both fed and fasted rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (IFFA CREDO, Les Oncins, France) weighing 220 g were kept at a constant temperature (21°) and subjected to a 11 hr light (9:00 a.m.–8:00 p.m. hr)–13 hr dark cycle. Food (a standard laboratory chow) and tap water were provided *ad lib.* Except from some groups of animals (see Results), the rats were food deprived for 24 hr before the onset of experiments.

Plasma metabolites measurements. Rats were killed by cervical dislocation. Blood was collected into heparinized tubes and immediately centrifuged whereas the liver was removed within 20 sec, plunged in liquid nitrogen and stored at −80° until TPO activity analysis. Each plasma sample was separated into subfractions. One of these subfractions was kept in ice for immediate measurement of total TRP concentration whereas the other subfractions were stored at −80° until analysis. Plasma total TRP was estimated by HPLC-UV [16]. Insulinemia and corticosteronemia were assessed by radioimmuno-

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assay kits, using respectively monoiodinated porcine [^{125}I]insulin as tracer and human insulin as standard (CEA, Gif-sur-Yvette, France) and [^{3}H]cortisol and cortisol binding protein (bioMerieux, Crappone, France). Plasma glucose levels were measured by the glucose oxidase method [17] using a glucose analyser (Beckmann Instruments Inc., Palo Alto, CA).

Determination of hepatic tryptophan pyrrolase activity. In rats, liver TPO exists under two forms: the holoenzyme and the apoenzyme, forms which are the active haem-containing form and the haem-free form, respectively. Indeed, the haem free form requires the addition of exogenous haematin for demonstration of its activity *in vitro* [18]. The activity of the enzyme was determined in liver homogenates by measuring the formation of kynurenine from L-TRP [18] in the absence (holoenzyme activity) or the presence (total enzyme activity; i.e. apoenzyme plus holoenzyme activity) of added haematin, according to the procedure of Badawy [19]. Apoenzyme activity was obtained by difference between total enzyme activity and holoenzyme activity. When necessary, the endogenous saturation of the enzyme with haem was expressed as the percentage saturation (100 × holoenzyme activity/total enzyme activity) [20].

Drugs. Insulin was dissolved in a 0.9% saline solution: saline or insulin (Novo, Actrapid) solutions were injected s.c. in the flanks; the volume of injection was 1 mL/kg.

Statistics. All data have been expressed as means ± SE. Differences between means were assessed using a two-way analysis of variance followed by Newman-Keuls Q-test, except for the dose–response data which were analysed by a one-way analysis of variance followed by Dunnett's *t*-test.

RESULTS

Effects of insulin on plasma TRP levels and liver TPO activity

Administration of increasing doses (1–3 I.U./kg) of insulin to 24 hr starved rats triggered a significant elevation in plasma TRP levels ($F = 4.63$; $P < 0.01$; Fig. 1), that was not associated with changes in either holoenzyme or total enzyme activity (Fig. 1).

Time course of the effects of insulin

The time course of the effects of a 2 I.U./kg dose of insulin given to 24 hr fasted rats is shown in Figs 2 and 3. Hyperinsulinemia, which was still measurable 1 hr after insulin administration ($F = 19.21$; $P < 0.001$; Fig. 2), was associated with hypercorticosteronemia ($F = 52.34$; $P < 0.001$; Fig. 2) and a significant increase in plasma TRP levels ($F = 10.4$; $P < 0.01$; Fig. 3). Both changes lasted 2 hr after insulin administration (Figs 2 and 3). Hypoglycemia was also evidenced for the first 2 hr which followed insulin administration (data not shown). Besides, liver TPO activity, but not the percentage of saturation of the enzyme (data not shown), was increased 3 to 4 hr after insulin treatment ($F = 25.15$; $P < 0.001$ and $F = 16.97$; $P < 0.001$ for the holoenzyme activity and the total enzyme activity respectively; Fig. 3).

Comparative effects of insulin in fed and fasted rats

The possibility that insulin could have short-term effects (i.e. within 60 min) was tested under conditions of basal and starvation-stimulated TPO activity. Starvation affected insulinemia ($F = 10.9$; $P < 0.001$; Table 1), glycemia ($F = 116.6$; $P < 0.001$; data not shown), corticosteronemia ($F = 3.03$; $P < 0.05$; Table 1), plasma TRP levels ($F = 10.72$; $P < 0.001$; Fig. 4), both holoenzyme and total enzyme activities ($F = 73.6$; $P < 0.001$ for the former and $F = 40.7$; $P < 0.001$ for the latter; Fig. 4) and increased the percentage of saturation of the enzyme ($F = 11.45$; $P < 0.001$; data not shown). Insulin administration (30 min before analysis) increased corticosteronemia in the fasted rats only ($F = 27.2$; $P < 0.001$; Table 1). Besides, plasma glucose levels decreased in both fed and fasted rats ($F = 564$; $P < 0.001$; data not shown). Moreover, plasma insulin levels were much lower in the insulin-treated fasted rats, respectively compared to the insulin-treated fed rats ($F = 78.9$; $P < 0.001$, for the interaction between insulin treatment and starvation; Table 1). Lastly, insulin administration had no effect on either plasma TRP levels (excepted from the 48 hr starved rats; $F = 15.45$; $P < 0.001$, for the interaction between insulin treatment and starvation; Fig. 4), liver TPO activity (Fig. 4) or the percentage of saturation of the enzyme (data not shown).

DISCUSSION

The present work has examined the effect of insulin administration on hepatic TPO activity *in vivo*. The results presented herein strongly suggest that the rise in plasma TRP levels that is triggered by insulin is not mediated by an inhibition of liver TPO activity.

In an attempt to study the dose-dependent influence of insulin on plasma TRP and liver TPO activity, increasing doses of insulin were administered to fasted rats. Our results do indicate that insulin administration increased (at least until the 3 I.U./kg dose) plasma TRP levels without any alteration of liver TPO activity. The failure of the 4 I.U. dose of insulin to increase plasma TRP is rather difficult to interpret but this result confirms previous findings [4, 21], i.e. that hypoglycemia, per se, is not responsible for the rise in plasma TRP.

In another series of experiments, the metabolic effects of insulin were analysed with respect to time. Thus, the time-course of the effects of a single 2 I.U./kg dose was biphasic. During a first phasis (i.e. 1 and 2 hr after insulin injection), liver TPO activity remained unaltered despite hyperinsulinemia (associated with hypercorticosteronemia) and a rise in plasma TRP concentration. Inversely, during a second phasis (i.e. 3 and 4 hr after insulin injection) liver TPO activity increased at times when plasma TRP returned toward its basal level. Inasmuch as corticosterone is believed to activate TPO [22], it could be that insulin-induced TPO activity was not direct but rather mediated by corticosterone. Hence, the observation that both apoenzyme and holoenzyme activities, but not the percentage of saturation by haem, were lately increased by insulin

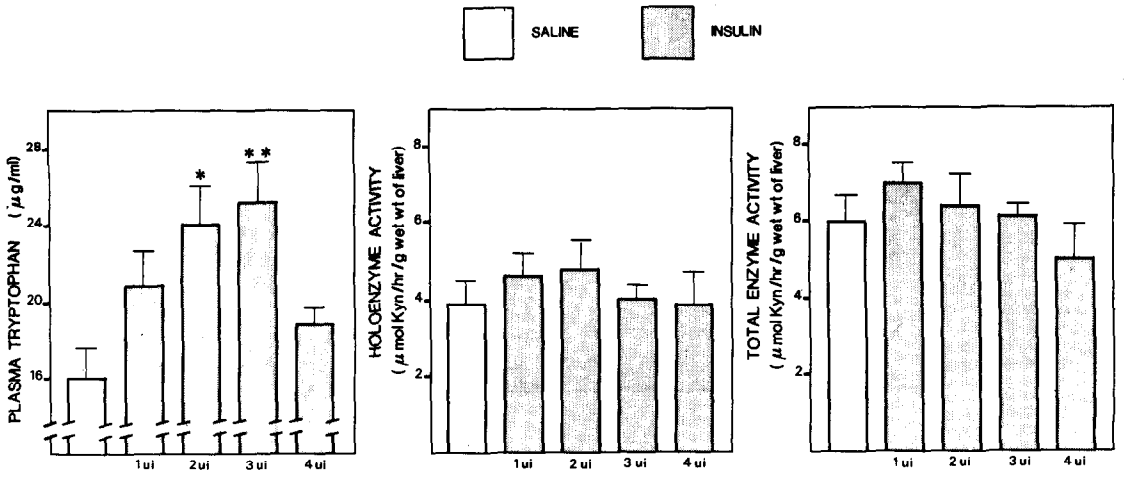

Fig. 1. Plasma tryptophan levels and liver tryptophan pyrrolase activity 1 hr after an injection of saline or insulin (1, 2, 3 or 4 I.U./kg, s.c.) to 24 hr-fasted animals. Holoenzyme activity (that measured in the absence of added haematin) and total enzyme activity (that measured in the presence of 2 μM haematin) are expressed in μmol of kynurenine formed per hr per g wet wt of liver. Each value is the mean ± SE of 4–5 animals. Differences between saline and insulin in each group are indicated as: * $P < 0.05$ and ** $P < 0.01$.

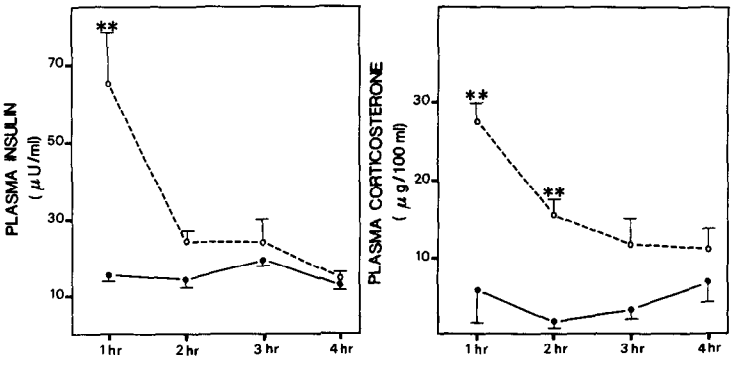

Fig. 2. Plasma insulin and plasma corticosterone levels at various times (1, 2, 3 and 4 hr) after the injection of saline (●) or insulin (○) (2 I.U./kg, s.c.) to 24 hr-fasted animals. Each value is the mean ± SE of 4–5 animals. Differences between saline and insulin in each group are indicated as: * $P < 0.05$ and ** $P < 0.01$.

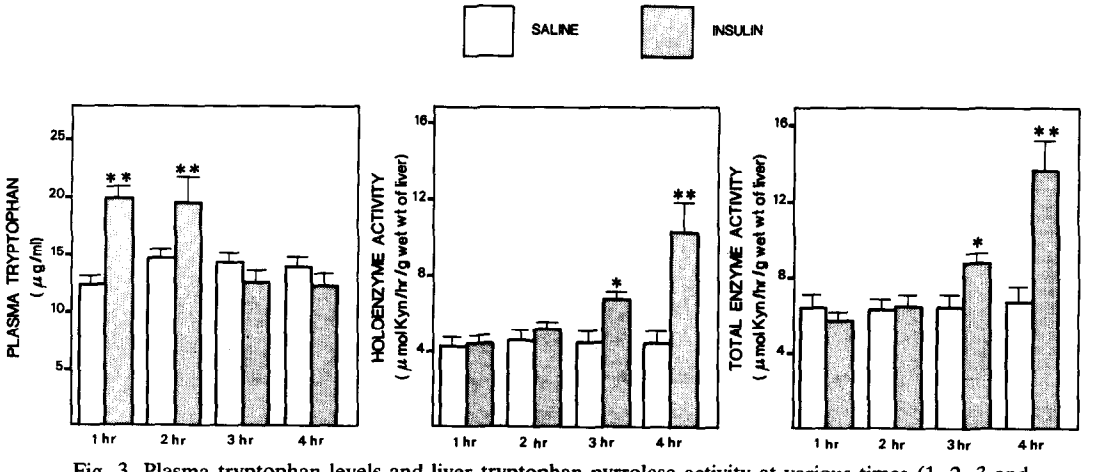

Fig. 3. Plasma tryptophan levels and liver tryptophan pyrrolase activity at various times (1, 2, 3 and 4 hr) after the injection of saline or insulin (2 I.U./kg, s.c.) to 24 hr-fasted animals. Holoenzyme activity (that measured in the absence of added haematin) and total enzyme activity (that measured in the presence of 2 μM haematin) are expressed in μmol of kynurenine formed per hr per g wet wt of liver. Each value is the mean ± SE of 4–5 animals. Differences between saline and insulin in each group are indicated as: * $P < 0.05$ and ** $P < 0.01$.

Table 1. Short-term effects of insulin administration on plasma insulin and corticosterone levels in fed and fasted rats

	Plasma insulin (μUnits/mL)	Plasma corticosterone (μg/100mL)
Fed		
Saline	25.3 ± 1.2	16.0 ± 4.7
Insulin	220.0 ± 20.7†	23.1 ± 4.5
24hrFasted		
Saline	13.6 ± 2.6	19.5 ± 5.1
Insulin	81.0 ± 26.0	37.8 ± 3.8*
48hrFasted		
Saline	18.0 ± 1.4	19.6 ± 2.5
Insulin	52.0 ± 13.0	34.7 ± 1.7*
72hrFasted		
Saline	19.0 ± 2.6	20.0 ± 3.0
Insulin	98.0 ± 30.8*	45.9 ± 5.5†

Plasma insulin and plasma corticosterone levels 30 min after the injection of saline or insulin (2 I.U./kg, s.c.) to fed or fasted animals (24, 48 or 72 hr of starvation). Each value is the mean ± SE of 5–6 animals. Differences between saline and insulin groups are indicated as: * $P < 0.05$ and † $P < 0.01$.

administration strongly suggested that (hypoglycemia-induced) hypercorticosteronemia [23] was actually the sole responsible for the increase in liver TPO activity [15, 24]. On the basis of the above mentioned data, it is suggested that the rise in liver TPO activity, that is elicited by hypercorticosteronemia, led to an increased TRP breakdown [25, 26] in the liver; the decline in plasma TRP levels observed throughout the second phasis would then be a consequence of this accelerated breakdown. On the other hand, it seems unlikely that hypercorticosteronemia could have interfered with a putative inhibiting effect of insulin during the first phasis since the induction of TPO by cortisol, even at high doses, does not occur before 2 hr after its administration to rats [27].

As a marked rise in plasma TRP was measured 1 hr after insulin administration (Fig. 2), the hypothesis that an inhibitory effect of insulin on TPO activity could have occurred before 1 hr was then tested. In addition, given that fasting has been reported to increase TPO activity [15] and to promote an up-regulation of insulin receptors in the liver [28–30], the short-term effects of insulin were analysed in rats submitted to increasing periods of food deprivation. Holoenzyme activity, total enzyme activity and the percentage of TPO saturation by haem were increased by starvation. Fasting-induced hypercorticosteronemia [15] and an important release of TRP from the liver, the latter being the result of protein catabolism [31] may both actively contribute to the high level of TPO activity and TPO saturation by haem in 48 and 72 hr fasted animals. Thus, whilst they confirm the activating effect of starvation on liver TPO activity [15], the results again indicate a lack of effect of insulin; thus neither TPO activity nor the percentage of saturation by haem were affected by insulin treatment. Moreover, excepted for the 48 hr starved rats in which plasma TRP decreased (for a reason that is still unclear) insulin administration did not elicit short term increases in TRP. Interestingly, in another model of liver TPO induction, i.e. that following TRP loading (80 mg/kg, 90 min beforehand), insulin administration did not affect TRP-induced TPO activation (data not shown).

The data presented herein bring evidence that insulin does not inhibit liver TPO activity *in vivo*,

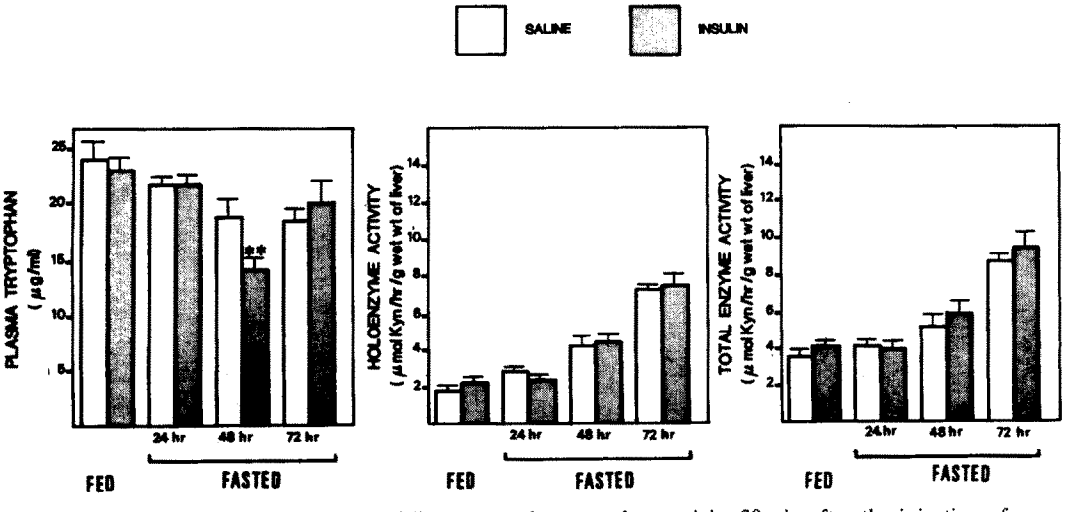

Fig. 4. Plasma tryptophan levels and liver tryptophan pyrrolase activity 30 min after the injection of saline or insulin (2 I.U./kg, s.c.) to fed or fasted animals (24, 48 or 72 hr of starvation). Holoenzyme activity (that measured in the absence of added haematin) and total enzyme activity (that measured in the presence of 2 μM haematin) are expressed in μmol of kynurenine formed per hr per g wet wt of liver. Each value is the mean ± SE of 5–6 animals. Differences between saline and insulin groups are indicated as: ** $P < 0.01$.

thus ruling out its mediation in the increase in plasma TRP observed in insulin-treated rats. The source of the additional TRP in plasma which appears after hyperinsulinemia requires further investigation.

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