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Effect of Insulin on Human Plasma Tryptophan and Nonesterified Fatty Acids (39945)

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Tryptophan is the only amino acid which exists in the blood in two forms, bound to albumin (the large majority) and free in solution (1). Current theory holds that tryptophan is bound to a single "indole-binding site" on the albumin molecule. Certain analogs of tryptophan and some drugs bind to this site competitively with tryptophan but these substances do not exist under physiological conditions and therefore do not constitute physiological control mechanisms for the binding.

Although there have been suggestions that there are other substances (2-4), the only class of substances which has been shown to affect the binding of tryptophan to albumin and which also exists under physiological conditions bound to albumin is nonesterified fatty acids (NEFA). In the rat (5-11) and pig (12) studies indicate that serum or plasma NEFA levels and free tryptophan levels are correlated positively. Furthermore, it has been shown that addition of NEFA to human serum or plasma *in vitro* causes a decrease in the amount of bound tryptophan and an increase in the amount of free tryptophan (7, 13). Therefore, it seemed reasonable to hypothesize that in humans, the levels of plasma bound and free tryptophan are regulated by NEFA levels.

As one test of this hypothesis, insulin, which is known to lower plasma NEFA levels, was administered to healthy, fasting humans during the intravenous insulin tolerance test (ITT). While these results are probably not generalizable to all conditions in humans, they do demonstrate that, under these conditions of insulin administration to fasting humans, neither free nor bound plasma tryptophan levels are correlated significantly with plasma NEFA levels.

Materials and methods. Subjects consisted of healthy volunteers between the ages of 36 and 52 years (mean age = 46 years) who were all within 10% of their ideal body weight and medication free. Three of the subjects were male and three were female.

The ITT was performed between 7 and 9 AM. All subjects were prohibited from eating, drinking, and smoking for 12 hr prior to the ITT. After resting for 1 hr, 0.1 unit of glucagon-free, regular U40 insulin (Iletin, Lilly) per kilogram body weight was administered through an indwelling catheter in an arm vein (14). Two 10-ml samples were removed at zero time (before insulin injection) and at 10, 20, 30, 45, and 60 min following insulin administration. Subjects remained resting during the entire procedure.

Each 10-ml heparinized sample was centrifuged immediately for 5 min at 7000*g* and 20°. The plasma was removed and allowed to remain at room temperature until all the samples were collected. The plasma samples were recentrifuged simultaneously for 10 min at 7000*g* and 20°, and divided in half, one aliquot being frozen immediately and the second aliquot being deproteinized and then frozen for later determination of free tryptophan. Deproteinization was accomplished by centrifugation for 30 min at 20° and 560*g* using Amicon CF-25 membrane cones. Total and free tryptophan were determined by the method of Denckla and Dewey (15) as modified by Lehmann (16) and Bloxam and Warren (17). Bound tryptophan levels were calculated by subtracting free tryptophan levels from total tryptophan levels. NEFA were determined by the method of Dole (18) as described by Chen (19). Total amino acids were determined by the method of Palmer and Peters (20). Albumin was determined by the procedure of Rodkey (21) using

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albumin standard and buffered albumin dye purchased from the American Monitor Corporation.

Results. Figure 1 shows the effect of insulin administration on free tryptophan (1A), total tryptophan (1B), NEFA (1C), and plasma amino acids (1D). The mean concentration of plasma free tryptophan did not change significantly with time (Fig. 1A), although there was a tendency for an increase at 10 min and a decline thereafter.

Total tryptophan increased slightly between 0 and 20 min and then decreased. Overall, there was only a slight decrease during the entire 60-min experimental period (Fig. 1B; $r = -0.36$, $n = 36$, $P < 0.05$), and only at 60 min was tryptophan significantly lower than the zero-time level ($P < 0.05$). The ratio of free to total tryptophan did not change significantly during the entire experimental period.

The amount of tryptophan bound to albumin (moles/mole of albumin) decreased during the hour after insulin administration ($r = -0.419$, $n = 34$, $P < 0.025$). The mean fasting bound tryptophan level was 0.074 ± 0.002 mole/mole of albumin (mean $\pm$ SEM).

In contrast, plasma amino acids decreased significantly following insulin administration (Fig. 1D; $r = -0.709$, $n = 35$, $P < 0.01$). At all times after 10 min, plasma amino acid levels were lower than zero-time levels. The value at 60 min was 79% of that at zero time.

The mean fasting NEFA level was 1.42 ± 0.16 mole/mole of albumin (mean $\pm$ SEM). Following insulin injection, plasma NEFA fell steadily for 30 min to approximately 50% of the zero-time value (Fig. 1C). Between 30 and 60 min, it rose slightly to about 57% of the zero-time value. Overall, the decrease was significant ($r = -0.539$, $n = 36$, $P < 0.001$) and NEFA levels from 20 to 60 min after insulin injection were all significantly lower than the zero-time value.

Figure 2 shows observed bound tryptophan plotted against bound NEFA for each subject at each time point. The slope of the regression line was slightly, although not significantly, positive ($r = 0.307$, $n = 34$, $P < 0.10$), although McMenamy and Oncley's *in vitro* findings indicate that bound tryptophan should *decrease* significantly as bound NEFA increases (1).

Discussion. Under the conditions of the ITT, as presented in this paper, free trypto-

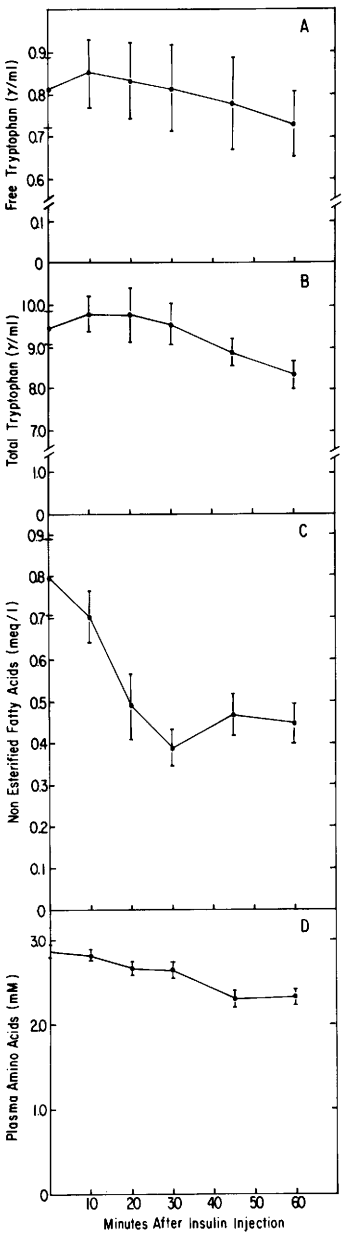

FIG. 1. Plasma levels of free tryptophan (A), total tryptophan (B), nonesterified fatty acids (C), and amino acids (D) at various times after injection of insulin. Values are represented by means $\pm$ standard errors of the mean (SEM).

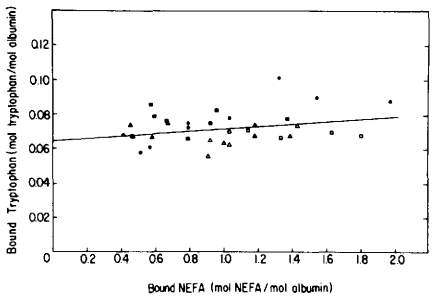

FIG. 2. Moles of tryptophan per mole of albumin plotted against moles of nonesterified fatty acids per mole of albumin. Each of the six symbols represents a single subject. Plasma samples were taken at various times before and during the insulin tolerance test.

phan plasma levels were not correlated with NEFA plasma levels. This does not preclude the likely possibility that NEFA may be one of the regulatory factors of plasma free tryptophan levels under certain physiological or pathological conditions in humans, e.g., conditions in which there are elevated NEFA plasma levels.

There have been studies in humans which suggest that plasma NEFA and free tryptophan levels are related. One minute after electroconvulsive therapy in depressed patients on medication, there is an increase in both plasma NEFA and free tryptophan levels, but no significant change in total tryptophan levels (22). Patients with fulminant hepatic failure have higher plasma levels of both free tryptophan and NEFA than patients with neurological diseases (23). Lipsett *et al.* (13) observed that after oral glucose administration to fasting humans, which is known to cause the release of a glucagon-like substance (24) as well as insulin, there is a decline in serum free tryptophan coincident with a decline in NEFA, but no significant change in bound tryptophan.

Other studies, besides the one presented in this paper, indicate that factors besides NEFA influence free tryptophan plasma levels. The percentage free tryptophan in plasma from fasting humans correlated significantly with NEFA plasma levels (r = 0.509) (25). Given a correlation coefficient of 0.509, we can say that 26% (r^2) of the variance of tryptophan can be explained by variation of NEFA levels, indicating that other factors must be involved. During tryptophan infusion in fasting humans, spontaneous increases occurred in both plasma NEFA and free tryptophan levels, but instances of either one varying independently of the other were noted (25).

With regard to insulin's effect on plasma or serum total tryptophan levels, insulin has been reported to cause no change in its levels in fasting normal humans at 1 hr after intravenous administration (26) and a decrease at 2 hr (27).

In contrast to this in fasting rats, insulin produced an increase in plasma or serum total tryptophan levels of 20–50% within 1 to 2 hr after its administration (6, 11, 28–30). This increase is observed whether the insulin is administered intraperitoneally, subcutaneously, or intravenously (28). However, insulin caused a large decrease in rat NEFA plasma levels (11) which is similar to its effect on NEFA in humans. The increase in total tryptophan levels of rat plasma or serum after insulin administration has been explained by the decrease in NEFA, which presumably frees the indole-binding sites on the albumin molecules for tryptophan attachment. This interpretation is supported by the fact that, concomitant with the increase in total plasma or serum tryptophan levels after insulin administration to rats, there is a decrease in free tryptophan levels (6, 11, 30).

Therefore, in the rat, insulin administration caused an increase in the total tryptophan plasma level, whereas in the human it either caused a decrease or did not alter the total tryptophan plasma level. These contrasting results indicate that the rat is not an adequate model to study the interrelationship between insulin and plasma tryptophan levels in humans. This point is especially important since the interrelationship of brain and plasma tryptophan levels has been studied extensively in the rat and the results have been extrapolated to humans. Since this blood–brain relationship cannot be studied directly in humans, it might be necessary to utilize another animal model [e.g., dogs, (31)] which more closely approximates humans with respect to plasma tryptophan response to insulin administration.

Summary. One hour after insulin admin-

istration to healthy, fasting humans, plasma nonesterified fatty acid (NEFA) levels decreased by approximately 40% of the preinjection value. Contrary to predictions, plasma free tryptophan did not change and plasma bound tryptophan decreased by 12% during the 1 hr after insulin administration. In these subjects, neither bound nor free plasma tryptophan levels are correlated significantly with plasma NEFA levels. These data clearly demonstrate that the levels of free or bound tryptophan in plasma are not regulated by changes in NEFA plasma levels in fasting humans after insulin administration.

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