Effect of Tianeptine on Glucose Tolerance with Insulin Secretion in Human: Potential Anti-Diabetic Effect of the Drug

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Abstract: Due to the fact that drugs which interfere with the serotonin (5-HT) uptake (doxepin, sertraline, fluvoxamine and fluoxetine), were able to minimize the insulin release from islet beta-cells and counteract hypoglycemia, we investigated the effects of tianeptine, a drug which enhances 5-HT uptake during the oral glucose tolerance test. We found that the drug triggered significant and sustained insulin rises which were opposite to the plasma glucose decreases. The fact that significant noradrenaline/adrenaline (NA/Ad) plasma ratio paralleled insulin rises throughout the test is consistent with the excitatory role played by the peripheral neural sympathetic activity. Additionally, the positive correlations registered between NA versus DA (dopamine) plasma levels indicate that the latter arose from sympathetic nerves rather than the adrenal glands. Furthermore, the significant reductions of both platelet and plasma 5-HT registered throughout the OGTT plus tianeptine test support the postulation that reduction of the parasympathetic drives is occurring. This parasympathetic drive is responsible for the secretion of 5-HT from the enterochromaffin cells. Finally, considering that circulating 5-HT excites alpha-cells and modulates beta-cells, it is possible to infer that minimization of this factor contributed to the insulinogenic effect displayed by the association of this drug to an oral glucose load. We also discussed the peripheral and central nervous system mechanisms responsible for the insulinogenic effect registered throughout the test.

Keywords: Diabetes, hyperinsulinism, neural sympathetic activity, serotonin, tianeptine.

INTRODUCTION

The concept of "entero-insular axis" began in 1962 when we demonstrated that the gastrointestinal (GI) hormone secretin was found to be able to excite the release of not only the pancreatic exocrine secretion but also the endocrine (insular) activity [1-3]. It was demonstrated that this gastrointestinal factor acted at the beta-cell level. In addition, it was also found that another gastrointestinal factor, serotonin (5-HT), was able to inhibit the insulin release *in vitro* [4,5]. The above findings were further ratified by multiple research studies [6,7]. Furthermore, whereas intraportal infusion of serotonin was able to inhibit the stimulated insulin secretion in dogs [5], insulin (subcutaneously injected) was able to elevate platelet serotonin (p5-HT) circulating levels in essential hypertensive patients but not in non-essential hypertensive or normal subjects [7]. This phenomenon was attributed to the hyperactivity of neural sympathetic system plus the hypoactivity of the adrenal glands secretion always observed in the essential hypertensive subjects. Moreover, many studies carried out by other researchers ratified our preliminary findings and even showed that not only serotonin but other GI factors were also able to control the secretion of insulin by beta cells [8]. The above information has been quoted in our review article discussing hyperinsulinism [9].

Other research studies carried out in our department demonstrated that the experimentally-induced depression (captivity) was able to provoke a diabetogenic effect in dogs [10]. The fact that these dogs showed greatly increased p5-HT, and that in addition normalization of the OGTT plus p5-HT levels paralleled both the disappearance of the psychological disorder as well as the diabetic syndrome; led us to postulate that serotonin played a primary role in the inhibition of the islet beta-cells [11,12]. Furthermore, we also demonstrated that the chronic administration of some dopaminergic (DA) blocking agents like sulpiride, were able to provoke a diabetogenic effect plus a p5-HT elevation similar to that registered during captivity [11]. Evenmore, the fact that normalization of clinical, hormonal and glucose parameters was observed after the interruption of drug administration allowed us to postulate that serotonin was the common etiopathogenic factor responsible for both the metabolic and the psychiatric disorder [13]. The above findings were furtherly confirmed and published [9,14,15]. Finally, our demonstration dealing with the annulment by doxepin (a serotonin uptake inhibitor) of the hyperinsulinism plus hypoglycemia syndrome, affecting a great bulk of patients [16] led us to assess the possible insulinogenic effect of drugs like tianeptine which enhances rather than inhibits the uptake of 5-HT [17].

MATERIALS AND METHODOLOGY

One OGTT plus placebo and one OGTT plus tianeptine test were carried out 2 weeks apart in 48 caucasic normal voluntary humans (26 males and 22 females), whose ages

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ranged from 20 to 63 years (Mean ± s.e.m. = 42.6 ± 5.8). All of them gave informed written consent and the procedure was approved by the ethical committee of FUNDAIME. All subjects were within 10% of ideal body weight, and none had undergone abdominal surgery or were taking any medications. None of the subjects had physical or psychiatric illness. Subjects were rated on a modified Hamilton Depression Rating Scale for Depression and all of them completed the self-rating Beck Depression Inventory [16] in order to discard subjects with depression. Pregnancy, lactation, smoking and/or alcoholism also excluded subjects.

Analytical Methods

Noradrenaline (NA), adrenaline (Ad), dopamine (DA), plasma free serotonin (f5-HT), platelet serotonin (p5-HT), glucose and insulin were measured throughout the 180 minute testing period. For all parameters, the samples were assayed in duplicate and all determinations were made simultaneously. We used reverse-phase, ion-pair high performance liquid chromatography with electrochemical detection for the detection of monoamines. Optimization of chromatographic conditions and attainment of adequate quantification parameters allowed us to maximize sensitivity and reproducibility.

All tests were performed on recumbent subjects, after 14 hours of fastening. A heparinized venous catheter was inserted into a forearm vein at least 30 min before beginning the tests. Blood samples were collected at 0, 60, 90, 120 and 180 minutes. Each subject drank a 30% glucose solution (1 gm/kg of ideal body weight) plus placebo or an oral dose of tianeptine (12.5 mg = one tablet). Blood for catecholamines and serotonin assays was transferred to plastic tubes, each containing 20 mg of EDTA plus 10 mg of sodium bisulfite/ml of solution. The tubes were carefully inverted and placed on ice. The blood was promptly centrifuged at 600 rpm for 15 min at 4° C in order to obtain platelet-rich plasma. Two milliliters of platelet-rich plasma, obtained for determination of platelet serotonin (p5-HT), were taken and stored at –70 °C until assayed. The remaining blood was again centrifuged at 7,000 rpm. The supernatant, platelet-poor plasma, was divided into two portions for determination of catecholamines and f5-HT, after which both portions were stored at –70 °C until assayed.

A physician in constant attendance noted any symptoms reported by the subjects and monitored heart rate, systolic blood pressure and diastolic blood pressure every 30 minutes.

Reagents and Standards

Noradrenaline, adrenaline, dopamine, serotonin creatinine sulphate, dihydroxybenzylamine, sodium octyl sulphate, dibutylamine, acid-washed aluminium oxide, Na2HPO4, citric acid and EDTA were purchased from Sigma-Aldrich (St Louis, MO, USA). Microfilters were purchased from Whatman Inc. (Florham Park, NY, USA) through Merck S.A, (Caracas, Venezuela). Acetonitrile and 2-propanol were obtained from Merck, S.A. (Caracas, Venezuela). Glass-distilled water was de-ionized and filtered through a Milli-Q reagent grade water system (Millipore, Bedford, MA, USA). Solvents were filtered through a 0.2 μm Millipore filter and were vacuum deaereated. Standard solutions (1 mmo1/1) were prepared in 0.1 mo1/1 perchloric acid and diluted to the desired concentration.

Equipment

Liquid chromatography was performed using Waters 515 HPLC pump (Waters Corporation, Milford, Massachusetts. USA) equipped with a Rheodyne valve injector 7125i, which was fitted with a 50 µl sample loop (Rheodyne; Berodine, Berkeley, CA, USA). A 15 cm x 4.6 mm inner diameter Discovery C18 column packed with octadecylsilane 5 μm particles was preceded by a column prefilter of 2 μm porosity, both from Supelco/Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). The detection system was a Waters 460 Electrochemical Detector (Waters Corporation, Milford, MA, USA). The potential of the working electrode (glass carbon) was set at + 0.61 V versus the Ag-AgCl reference electrode for the detection of catecholamines and 0.70 V versus the Ag-AgCl for the detection of indoleamines. The chromatograms were registered and quantified with the Empower software from Waters Corp. The results were corrected for the volume of EDTA added.

Analytical Assays

Plasma catecholamines. The assay was performed by extraction of the catecholamines onto 20 mg of alumina followed by their elution with 200 µl of 1.0 mo1/L $HClO_4$ using Regenerated Cellulose microfilters 0.2 μm pore size (Whatman Inc). We calibrated the instrument with standard plasma: after incubation with acid-washed aluminum oxide, a plasma pool of free catecholamines was processed similarly to plasma samples, but 20 μl of a standard solution of NA, Ad and DA (50, 25 and 25 ng/ml, respectively) were added to the plasma pool. Both the standard plasma and the sample plasma were supplemented with 20 µl of internal standard (100 ng/ml of dihydroxybenzylamine). The mobile phase was KH_2PO_4 6.8045 g/L, EDTA 0.100 gm/L and di-N-butylamine 100 µl/L. Sodium octyl sulphate was added as ion-pair agent in a concentration of 0.6125 g/L with the pH adjusted to 5.6. The sensitivity of this method for NA, Ad and DA was 6.4, 5.8 and 2.0 pg/ml, respectively. The intra-assay coefficients of variation were 2.8, 4.0 and 4.0 %, respectively. The inter-assay coefficients of variation were 6.7, 4.5 and 4.3 %, respectively.

Plasma indoleamines. After sonication of platelet-rich plasma to disrupt the platelets (Ultrasonic Liquid Processor, model 385; Heat Systems Ultrasonics Inc., Farmingdale, NY, USA), both platelet-rich and platelet-poor plasma were processed in the same way: 200µl of 3.4 mol/L perchloric acid and 50 µl of 5-hydroxy-tryptophan solution (114.5 µg/m1) as internal standard, were added to 1 ml of plasma vortexed and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was filtered through a 0.22 µm membrane (Millipore) and 10 µl was injected into the column. Calibration runs were generated by spiking blank platelet-poor plasma with 50 µl of a solution containing 5-HT (10 µg/ml) and 50 µl of 5-hydroxy-tryptophan (114.5 µg/ml). This standard plasma was processed in the same manner as the samples. The mobile phase was citric acid 3.8424 gr/L, sodium acetate 4.1015 gr/L, EDTA 0.100 gr/L, di-N-butylamine 100 µl/L and 30 ml/L of 2-propanol. Sodium octyl sulphate was added as ion-pair agent in a concentration of 4.25 mg/L with a pH of 5.0. The sensitivity of the method
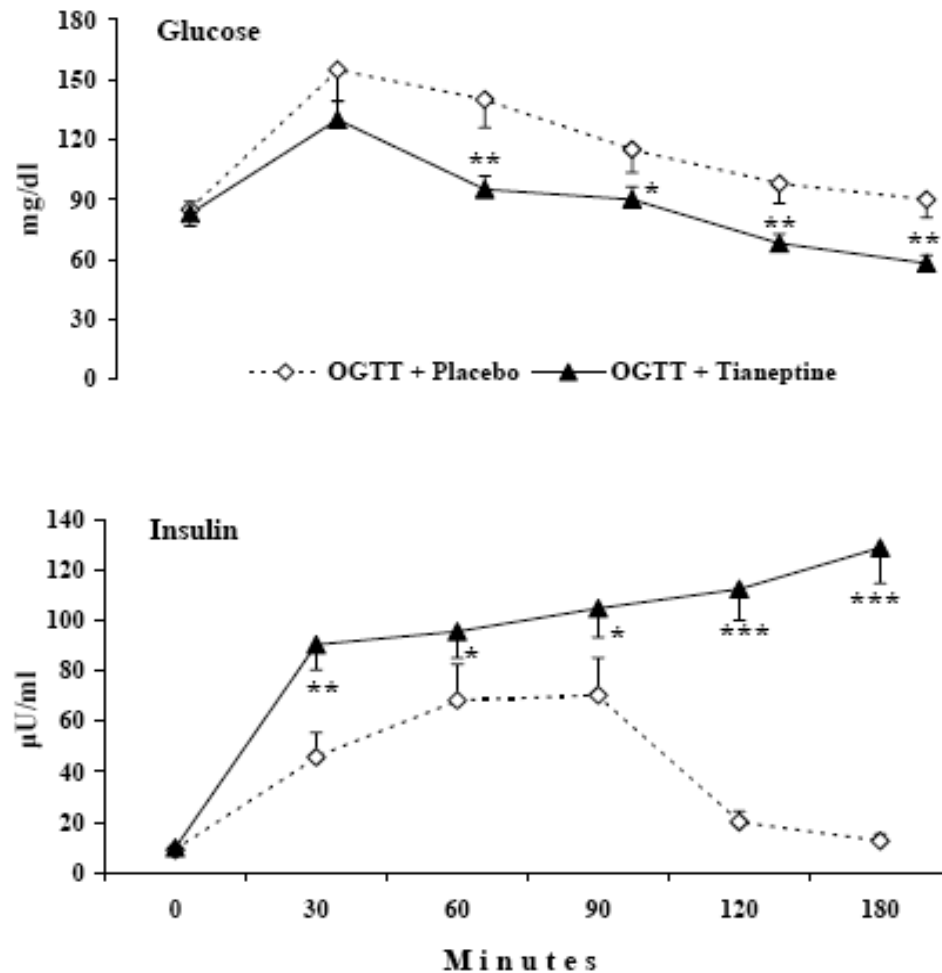
Fig. (1). The addition of tianeptine to an oral glucose load potentiated both the insulin rises plus its hypoglycemic effects normally registered throughout this test without the drug. (n = 48) Values are expressed as mean ± s.e.m. (*) $p<0.05$; (**) $p<0.02$; (***) $p<0.01$ (OGTT + Tianeptine versus OGTT + Placebo).

for serotonin was 0.1 ng/ml. The intra-assay coefficients of variation for p5-HT and f5-HT were 6.2 and 8.7%, respectively.

Plasma insulin. This was determined by a radioimmunoassay with an insulin-antibody precipitate [16].

Plasma glucose. The levels of plasma glucose were estimated by enzyme-linked method in an auto chemistry analyzer (Rayto, model 1904C, Rayto Life and Analytical Sciences, Chine).

Statistical Methods

Results are expressed as mean ± SE. Multivariate analysis of variance with repeated measurements, paired t test, and correlation coefficients (exploratory factor analysis) were used; $p < 0.05$ was considered significant. Dbase Stats (TM) by Ashton Tate and Statview SE + Graphics by Abacus were used for statistical analyses.

RESULTS

Oral Glucose + Placebo Test

The assessment of circulating neurotransmitters after an oral glucose load carried out according to the present protocol ratified other previously published research studies

[18]. Only NA and p5-HT circulating values showed significant and sustained rises that paralleled insulin increases and opposed plasma glucose reductions. In addition, Ad and DA showed significant decreases at the 90min, 120, and 180min periods. All subjects fell asleep and displayed rapid eye movements (REM) during the test. Maximal rise of glucose was registered at the 30min period, whereas minimal mean values were reached at the 180min period. In addition to the above, although both NA and p5-HT levels were raised after the oral glucose load, the NA/p5-HT ratio showed significant decreases throughout the OGTT.

All subjects showed a normal OGTT. Normal rises of glucose and insulin were registered throughout the test (Fig. **1**). Aside from glucose rise, significant increases in NA (Fig. **2**), NA/Ad (Fig. **3**), and p5-HT (Fig. **4**) were registered. Significant reduction of Ad values were registered throughout the test. No significant changes were observed in DA and f5-HT. Slight but significant decreases of heart rate and systolic blood pressure (SBP) mean ± s.e.m. values were registered throughout the test. Significant reductions in the NA/p5-HT (Fig. **3**), Ad/p5-HT and DA/p5-HT ratios mean values were also registered at 60, 90, 120 and 180min (Fig. **5**). All of our subjects fell asleep and displayed REM during
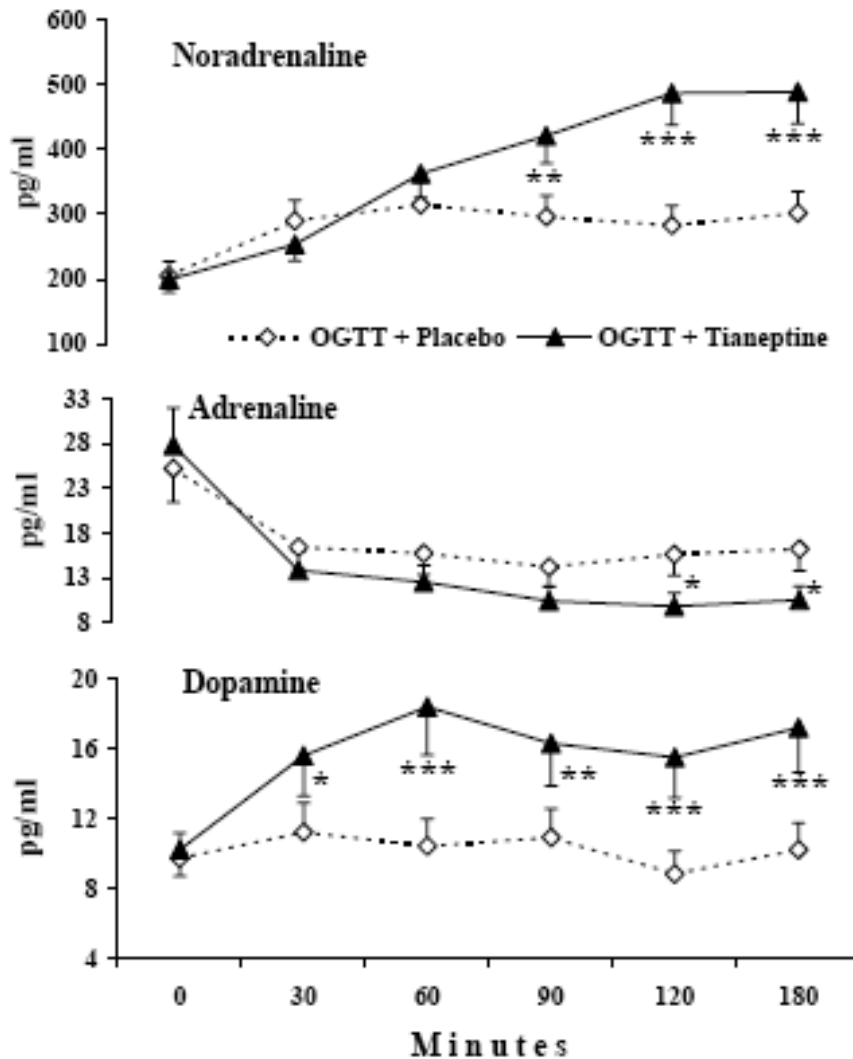
Fig. (2). The addition of tianeptine to an oral glucose load potentiated the normal NA and DA rises (neural sympathetic activity) as well as the decreases of Ad (adrenal sympathetic activity), always registered troughout this test without the drug. (n = 48) Values are expressed as mean ± s.e.m. (*) $p<0.05$; (**) $p<0.02$; (***) $p<0.01$ (OGTT + Tianeptine versus OGTT + Placebo).

OGTT, especially accentuated at these same periods (90 and 180min). Correlations are in Table **1**.

Oral Glucose + Tianeptine Test

Subjects did not show drowsiness throughout the test. This fact, in contraposition with the other one occurred in the glucose challenge alone, may be associated with the decreased circulating levels of serotonin observed with the addition of tianeptine. The potentiation of insulin secretion triggered by a small dose of oral tianeptine when added to an oral glucose dose (Fig. **1**), should be associated with the changes of the autonomic nervous system parameters registered in this study, namely, the increase of both NA and DA plus the decreases of Ad (Fig. **2**), p5-HT and f5-HT circulating levels (Fig. **4**). With respect to this, we will discuss the possible physiological and pharmacological mechanisms underlying the above findings.

Plasma Catecholamine Changes

The close positive correlations registered between NA and NA/Ad ratio when these parameters were plotted versus

DA strongly suggest that the latter arose from the sympathetic nerves rather than the adrenal glands (Table **2**). Furthermore, the significant positive correlations registered between NA and DA versus diastolic blood pressure (DBP), but not versus SBP and heart rate (Table **3**), indicates that neural not adrenal sympathetic activity was responsible for these changes.

Circulating Indolamine Changes

The significant reductions of both p5-HT and f5-HT indicate that the normal post-glucose enhancement of these indoleamines was reversed by the drug (Fig. **4**). No significant correlations were found between these parameters when plotted against catecholamines before or after the administration of the glucose load.

Plasma Glucose Changes

Small but significant rise of glucose levels were registered at the 30min and 60min post-glucose periods. Non significant increases were found at the 90min, 120min and 180min post-glucose periods (Fig. **1**).
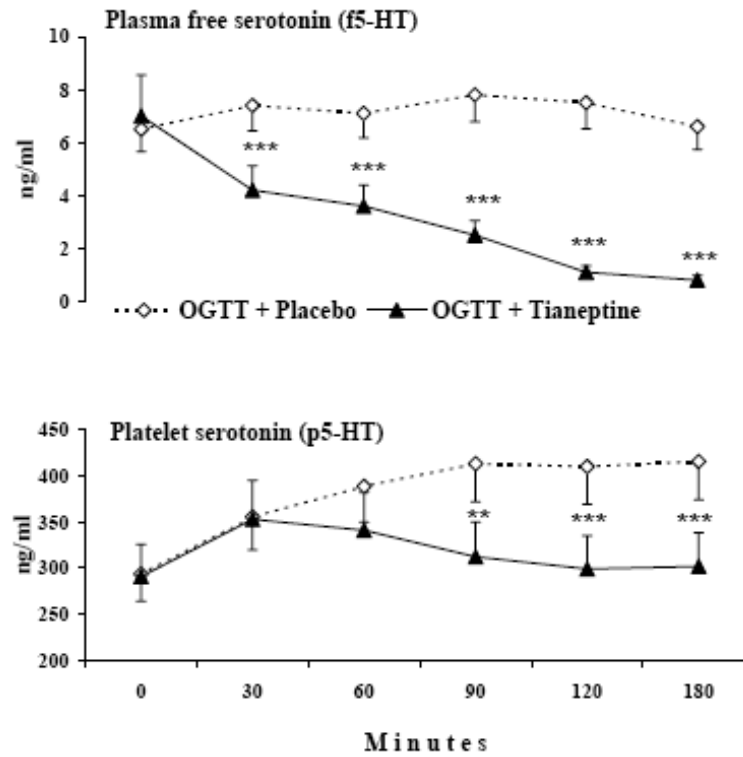
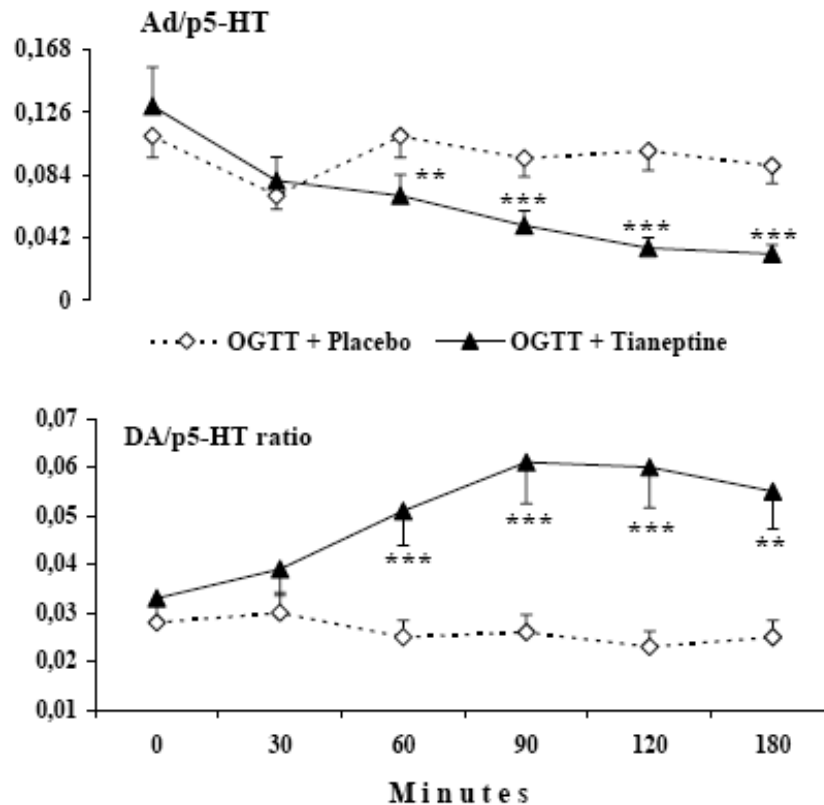**Fig. (3).** The addition of tianeptine to an oral glucose load reversed the plasma serotonin (f5-HT) and platelet serotonin (p5-HT) rises, normally registered troughout this test without the drug. (n = 48) Values are expressed as mean ± s.e.m. (**) $p<0.02$; (***) $p<0.01$ (OGTT + Tianeptine versus OGTT + Placebo).
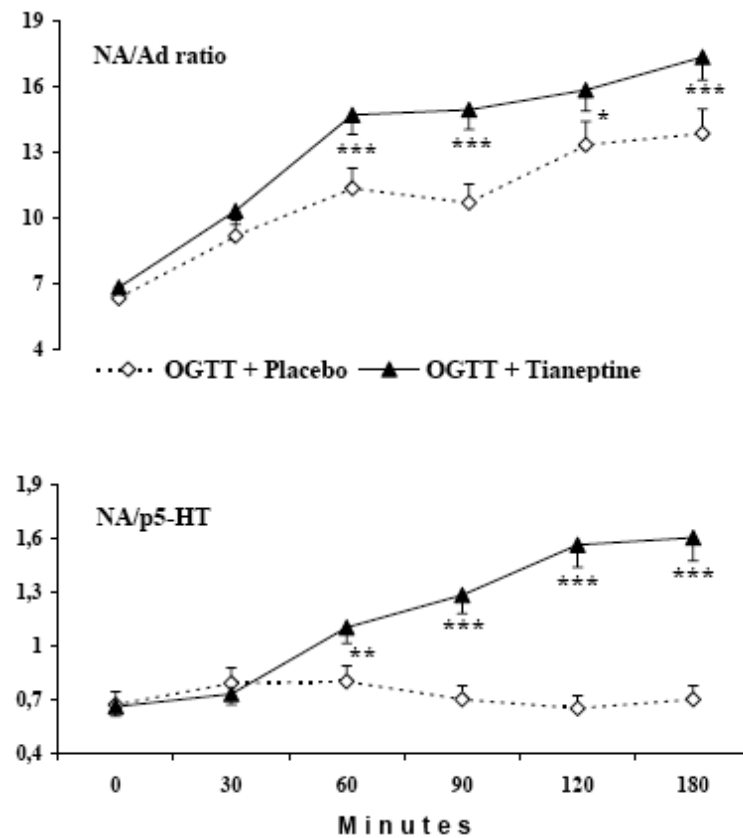
Fig. (4). The addition of tianeptine to an oral glucose load potentiated the normal NA/Ad ratio registered throughout this test without the drug. It also provoked the enhancement of the NA/p5-HT ratio triggered by both the NA rises and the p5-HT fall. (n = 48) Values are expressed as mean ± s.e.m. (*) $p<0.05$; (**) $p<0.02$; (***) $p<0.01$ (OGTT + Tianeptine versus OGTT + Placebo).

Fig. (5). The addition of tianeptine to an oral glucose load triggered the fall of both adrenaline (Ad) and platelet serotonin (p5-HT) as well as the Ad/p5-HT ratio. In addition, significant increases of the DA/p5-HT ratio were observed. (n = 48) Values are expressed as mean ± s.e.m. (**) $p<0.02$; (***) $p<0.01$ (OGTT + Tianeptine versus OGTT + Placebo).

lasma Insulin Changes

Significant, progressive and sustained insulin rises were registered starting with the first 30min period until the final 180 min period (Fig. **1**).

Correlations

Significant positive correlations were noted between NA versus DA; NA/Ad versus DA; NA/Ad versus DBP; NA versus insulin; NA/Ad versus insulin; and DA versus insulin at the 60min, 90min 120min and 180 min periods. Significant negative correlations were found between DA versus DBP at the last four periods (Table **2**).

DISCUSSION

The potentiation of the insulin secretion triggered by the addition of a small dose of tianeptine to an oral glucose load should be associated with the enhancement of neural sympathetic activity registered in this study as inferred from the close positive and negative correlations we found between NA versus DA and NA versus Ad, respectively, as well as from the significant positive correlations demonstrated between NA versus insulin and DA versus insulin. Conversely, significant negative NA versus p5-HT and DA versus p5-HT correlations were registered during the post-glucose + tianeptine trial. These findings fit well with the progressive and significant reduction of p5-HT and increases of NA and DA values registered throughout the trial. In addition, it may explain the absence of sleep

registered in this group. This fact contrasts with other one showing that the oral glucose challenge provokes sleep in normal subjects. This effect is positively correlated with the increase of circulating serotonin, always registered in them [18]. On the other hand, the maximal fall of both Ad and f5-HT values interfered with the statistical assessment dealing with these parameters. These effects should be associated with the well known fact that insulin crosses the blood brain barrier and excites the A5(NA) neurons. These latter are responsible for the peripheral neural sympathetic activity which exerts hypoglycemic effects at peripheral level.

Other significant positive correlations demonstrated between NA and NA/Ad versus DBP as well as the significant negative correlation found between DA versus DBP allow us to postulate that the increases of both catecholamines depend on the enhancement of neural sympathetic activity provoked by the OGTT + tianeptine challenge.

With respect to the above and in order to understand why the association of tianeptine, a drug which reduces neural sympathetic activity [17], was able to enhance the normal neurosympathetic drive always registered throughout the OGTT should be carefully discussed.

Peripheral Mechanisms

Serotonin released from the enterochromaffin cells (ECC) during postprandial parasympathetic drives [19,20] modulates both beta and alpha cells [21]. It is incorporated

Table 1.   Correlations (r) Found During the Oral Glucose Tolerance + Placebo Test Performed in 48 Normal Voluntary Humans (26 Males and 22 Females)

	PERIODS					
	0min	30min	60min	90min	120min	180min
NA *vs* Ad	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA *vs* DA	n.s.	n.s.	.203*	.256*	.321**	.293**
Ad *vs* DA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA *vs* Glycemia	n.s.	n.s.	-.221*	-.325**	-.442**	-.456**
DA *vs* Gycemia	n.s.	n.s.	-.211*	-.247*	-.354**	-.376**
Ad *vs* Glycemia	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA *vs* p-5-HT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ad *vs* p-5-HT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DA *vs* p-5-HT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p-5-HT *vs* Glycemia	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA *vs* Insulin	n.s.	n.s.	.325*	.411**	.401**	.397**
DA *vs* Insulin	n.s.	n.s.	.214*	.222*	.216*	.217*
Ad *vs* Insulin	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p-5-HT *vs* Insulin	n.s.	n.s.	-.212*	-.223	-.231*	-.214*
NA *vs* HR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ad *vs* HR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DA *vs* HR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA/Ad *vs* SBP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA/Ad *vs* DBP	n.s.	n.s.	.207*	.214*	.221*	.218*
DA *vs* SBP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DA *vs* DBP	n.s.	n.s.	-.213*	-.210*	-.220*	-.222*

Levels of significance: (*) $p < 0.02$; (**) $p < 0.01$. n.s. = non significant. NA = noradrenaline, Ad = adrenaline; DA = dopamine; p-5-HT = platelet serotonin; HR = heart rate, SBP = systolic blood pressure; DBP = diastolic blood pressure.

into the insulin granules of the former [22,23], at which level exerts a modulatory effect [24-27]. Conversely, 5-HT facilitates the secretion of glucagon from the alpha cells into the blood stream [21,28-30]. Both serotonin and glucagon trigger the secretion of Ad from the adrenal glands and they excite hepatic glucogenolysis which counteract the hypoglycemic effect of the insulin released from beta cells. Thus, the overwhelming insulinogenic effect triggered by the addition of tianeptine to an oral glucose load should annul the alpha-cell plus adrenal gland cascade.

Findings showed that serotonin loaded into the beta cells localizes to the insulin secretory granules and is additionally co-released with it [26,27]. These authors demonstrated that there is little exocytotic activity of insulin + 5-HT at the basal glucose level, but increasing the sugar concentration results in increased insulin release from perfused beta cells. Furthermore, Deeney *et al*. [26] concluded that serotonin might be considered as a marker for insulin release. Thus, taking into account that tianeptine enhances the uptake of 5-HT by both neurons and platelets, it might also facilitate this mechanism at the beta cell level, which minimized the insulin release from the latter. Thus, this modulatory mechanism would be favored by the drug.

In addition to the above and taking into account that plasma serotonin excites both alpha-cells and adrenal glands directly [21,29,31-34], the disappearance of those hyperglycemic factors would favor the predominance of the

insulinogenic effect registered in this study. This phenomenon should be associated with other findings showing that adrenalectomy is able to interfere with the glucagon induced plasma glucose rises provoked by intravenous administration of 5-HT [35]. Finally, the fact that methysergide, a 5-HT antagonist, can prevent the glucagon effects, affords additional support to the postulation that serotonin was the responsible factor for the above phenomenon by acting at the islet cells directly [28,36].

The above comments are reinforced by others showing that intraperitoneally injected serotonin increases glucagon plus glucose and reduces insulin plasma levels; thus, the annulment of these effects triggered by the addition of tianeptine to the oral glucose load should be attributed to the interference by the drug, not only at CNS, but also at the peripheral level. In addition, the fact that adrenodemedullation prevented both the glucose rise and the insulin fall, but not the glucagon rise, indicated that the effects at the beta-cell level were mediated by the adrenaline released from the adrenal glands [21,29,31,33,34,37,38].

The peripheral neural sympathetic over-activity registered during the glucose plus tianeptine trial fits well with the minimization of the parasympathetic drive which excites the ECC system, as inferred by the lowering of p5-HT circulating levels observed throughout the test. This parasympathetic mechanism is bridled by sympathetic

Table 2.   Correlations (r) Found During the Oral Glucose Tolerance + Tianeptine Test Performed in 48 Normal Voluntary Humans (26 Males and 22 Females)

	PERIODS					
	0min	**30min**	**60min**	**90min**	**120min**	**180min**
NA *vs* Ad	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA *vs* DA	n.s.	n.s.	.368**	.377**	.405**	.426**
Ad *vs* DA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA *vs* Glycemia	n.s.	n.s.	-.267*	-.338**	-.422**	-.439**
DA *vs* Glycemia	n.s.	n.s.	-.332*	-.365*	-.411**	-.395**
Ad *vs* Glycemia	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA *vs* p5-HT	n.s.	n.s.	-.244*	-.332*	-.326*	-.299*
Ad *vs* p5-HT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DA *vs* p5-HT	n.s.	n.s.	-.237*	-.314*	-.333*	n.s.
p-5-HT *vs* Glycemia	n.s.	n.s.	.244*	.338*	.352*	.341*
NA *vs* Insulin	n.s.	n.s.	.211*	.253*	.324**	.323**
DA *vs* Insulin	n.s.	n.s.	.199*	.218*	.235*	.239*
Ad *vs* Insulin	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p-5-HT *vs* Insulin	n.s.	n.s.	-.368**	-.387**	-.359**	-.371**
NA *vs* HR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ad *vs* HR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DA *vs* HR	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA/Ad *vs* SBP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA/Ad *vs* DBP	n.s.	n.s.	.232*	.229*	.239*	.241*
DA *vs* SBP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DA *vs* DBP	n.s.	n.s.	-.218*	-.222*	-.219*	-.231*

Levels of significance: (*) $p < 0.05$; (**) $p < 0.02$. n.s. = non significant. NA = noradrenaline, Ad = adrenaline; DA = dopamine; p5-HT = platelet serotonin; HR = heart rate, SBP = systolic blood pressure; DBP = diastolic blood pressure.

nerves. With respect to this, it should be known that f5-HT but not p5-HT is able to excite the medullary area postrema (AP), which sends modulatory axons to both vagal and adrenergic medullary nuclei, responsible for the parasympathetic and adrenal sympathetic peripheral activities, respectively [20,39-41]. These activities were minimized throughout the OGTT + tianeptine test because of the fall of f5-HT. With respect to this, it should be known that both circulating Ad and ACh are able to increase f5-HT, the former because it triggers platelet aggregation, whereas the latter throughout the interference with the platelet uptake of serotonin [42].

In short, the annulment of both adrenal sympathetic and parasympathetic activities registered in this study should be associated to the suppression of the stimulatory drives by f5-HT at the area postrema, which sends excitatory and inhibitory axons to the medullary vagal complex and the C1(Ad) nuclei, respectively. The latter resulted in the minimization of the peripheral adrenal sympathetic activity registered in this study [43,44]. With respect to the above, it should be known that the C1(Ad) nuclei interchanges inhibitory axons with A5(NA) pontomedullary nucleus, responsible for neural sympathetic activity [45,46] thus, the

Table 3.   Changes in Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Heart Rate (HR) Induced in 48 Normal Voluntary Humans (26 Males and 22 Females) when One OGTT Plus Placebo and One OGTT Plus Tianeptine Tests were Carried Out 2 Weeks Apart

	OGTT + Placebo Test		OGTT + Tianeptine Test	
	0min	**180min**	**0min**	**180min**
HR (beats/min)	71 ± 5	$66 \pm 7*$	73 ± 7	$65 \pm 8**$
SBP (mmHg)	127 ± 8	122 ± 3	127 ± 8	124 ± 7
DBP (mm Hg)	73 ± 5	$76 \pm 4*$	71 ± 6	$88 \pm 4**$

Results are expressed as mean $\pm$ s.e.m. (*) $p < 0.05$; (**) $p < 0.02$.

disappearance of the activity of the former, triggered by tianeptine minimized the peripheral adrenal sympathetic drive and favored the absolute predominance of the neural sympathetic activity registered in the present study. With respect to the latter, exhaustive evidence has demonstrated that insulin crosses the blood brain barrier and excites the A5(NA) nucleus responsible for the activity of sympathetic nerves [9,47-49].

It is well known that at CNS level, tianeptine triggers the absolute disappearance of 5-HT from synaptic clefts. With respect to this, it should be known that the C1(Ad) medullary nuclei responsible for the adrenal glands activity [44] receive excitatory 5-HT axons from the dorsal raphe DR(5-HT) nucleus [50,51] and also from the medullary serotonergic nuclei: raphe magnus, raphe obscurus and raphe pallidus [52]. Thus, the drug should interfere with these excitatory drives to the C1(Ad) nuclei. In addition, exhaustive evidence has demonstrated that serotonin released at the hypothalamic level is responsible for the neuroendocrine cascade which excites the adrenal glands secretion thus; this excitatory adrenergic drive should also be suppressed by tianeptine [51].

In summary, considering that 5-HT is taken up by serotonergic axons, platelets and beta cells, tianeptine, a drug which enhances this mechanism, should eliminate this serotonergic hyperglycemic effect, which interfere with the insulin secretion. This postulation fits well with the ability of drugs that interfere rather than enhance 5-HT uptake (like doxepin, sertraline, paroxetine or fluvoxamine) to counteract hyper-insulinism + hypoglycemia syndrome [16,53].

We should also comment the DA plasma rises registered in this study. These findings are in line with our preliminary research studies showing that the diabetogenic effect triggered by captivity and/or sulpiride was positively and negatively correlated with 5-HT and DA blood levels, respectively [11-13]. This circulating neurotransmitters profile contrasts with the effects reported in this study. Furthermore, the significant NA versus DA positive correlations shown in the present research work indicates that both catecholamines have arisen from the sympathetic nerves. Finally, these findings agree with others showing that DA plays a direct excitatory role at the beta-cells level [54].

At the CNS level, findings showed that both insulin and glucagon crosses the blood brain barrier and excite the A5(NA) [55] and the C1(Ad) [56] pontomedullary nuclei responsible for the neural and adrenal sympathetic activities, respectively thus, the predominance of the former mechanism registered in the present study is in accordance with the above.

Summarizing, both insulin and glucagon crosses the blood brain barrier and excites the C1(Ad) medullary nuclei and the A5(NA), respectively. Both nuclei interchange inhibitory axons [9,45]. Predominance of the former would result in neural sympathetic activity plus hyperinsulinism + hypoglycemia whereas, the opposite peripheral profile depends on the overactivity of the C1(Ad) nuclei (adrenal sympathetic excitation plus hyperglycemia). This catecholaminergic binomial CNS axis is modulated by the CNS serotonergic axons arising from the pontine dorsal raphe (DR), median raphe (MR) nuclei and the medullary serotonergic system which includes the raphe magnus, raphe obscurus and raphe pallidus nuclei. A great bulk of quoted evidence has demonstrated that whereas the DR(5-HT) is positively correlated with adrenal sympathetic activity, the MR(5-HT) potentiates the neural sympathetic branch [52]. The enhancement of neural sympathetic activity + hyperinsulinism registered in the present study strongly suggests that the drug suppressed the activity of the DR(5-HT) + C1(Ad) axis which favored the disinhibition of the A5(NA) plus the MR(5-HT) binomial [57]. Thus, the potentiation by tianeptine of the normal glucose-induced neural sympathetic activity should be added to the minimization of both the parasympathetic plus the adrenal sympathetic activities which redound in the absolute predominance of the neural sympathetic system.

CONCLUSIONS

Type-2 diabetes depends on the unbalance between insulin and glucagon, at peripheral level. However, considering that both hormones cross the blood brain barrier and excite the C1(Ad) and the A5(NA) nuclei, respectively, we postulated that an adequate neuropharmacological manipulation at the CNS level would facilitate the predominance of the latter over the former favoring the hypoglycemic effect of the insulin. In accordance with this hypothesis, we were able to demonstrate that tianeptine, a drug which reduces serotonin levels at both plasma and synaptic clefts, was able to enhance plasma insulin throughout the oral glucose tolerance test in normal subjects. The facts showing that the plasma NA rises and blood serotonin decreases showed significant positive and negative correlations with insulin levels, respectively, allowed us to postulate that tianeptine should be a powerful neuropharmacological drug to treat diabetic patients.

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