Tianeptine Enhances Insulin Secretion Throughout the Oral Glucose Tolerance Test

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ABSTRACT

Drugs that inhibit the uptake of serotonin at the synaptic level, such as doxepin, are able to counteract hyperinsulism-induced hypoglycemia. Thus, we postulated that tianeptine, a drug which facilitates the uptake of serotonin at both synaptic and platelet levels, might display an antidiabetogenic effect. We investigated the oral glucose tolerance test (OGTT) + placebo in 38 normal humans. A second OGTT + tianeptine was performed 2 weeks later in the same subjects. We found that tianeptine potentiated a significant and sustained increase of insulin registered during the OGTT, further evidenced by a decrease in plasma glucose. Significant increases of the plasma noradrenaline (NA)/adrenaline (Ad) ratio paralleled insulin rises. Additionally, the positive correlation observed between the NA/Ad plasma and insulin levels is consistent with the well-known fact that insulin crosses the blood-brain barrier and excites the central nervous system (CNS)-noradrenergic neurons responsible for peripheral sympathetic nerve activity. Furthermore, significant reductions of both circulating serotonin (plasma serotonin plus platelet serotonin) registered throughout the tianeptine + glucose challenge. This observation supports the postulation that the drug interferes with the normal peripheral parasympathetic activity demonstrated throughout the OGTT. This hypoparasympathetic effect triggered by the drug is responsible for the hyposecretion of serotonin from the enterochromaffin cells. In conclusion, tianeptine potentiates the insulinogenic effect of OGTT throughout both CNS and peripheral mechanisms. Both effects depend on the drug's interference at

***Table 1**. Correlations (r) found during the OGTT + placebo (P) test.*

	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. Gl	n.s.	n.s.	-.221*	-.325**	-.442**	-.456**
DA vs. Gl	n.s.	n.s.	-.211*	-.247*	-.354**	-.376**
Ad vs. Gl	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. Gl	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA vs. Ins	n.s.	n.s.	.325*	.411**	.401**	.397**
DA vs. Ins	n.s.	n.s.	.214*	.222*	.216*	.217*
Ad vs. Ins	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p-5-HT vs. Ins	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: (*) $p < 0.02$; (**) $p < 0.01$. NA = noradrenaline, Ad = adrenaline; DA = dopamine; p-5-HT = platelet serotonin; HR: heart rate, SBP= systolic blood pressure; DBP = diastolic blood pressure.

CNS plus peripheral levels..

INTRODUCTION

The concept of "entero-insular axis" originated in 1962, when it was demonstrated that gastrointestinal (GI) hormone secretion was able to excite the release of not only pancreatic exocrine secretion, but also endocrine (insular) activity.[1-3] Thus, this gastrointestinal factor acts at the beta-cell level. In addition, it was found that another gastrointestinal factor, serotonin (5-HT), was able to inhibit insulin release in vitro.[4,5] The above findings were further ratified by multiple research studies.[6,7] Furthermore, researchers also found that intraportal infusion of serotonin inhibits stimulated insulin secretion in dogs.[5] Subcutaneously injected insulin was able to elevate the circulating levels of platelet serotonin (p-5-HT) in essential hypertensive patients, but not in non-essential hypertensive or normal subjects.[7] This phenomenon was attributed to the hyperactivity of the neural sympathetic system, plus the hypoactivity of the secretion of adrenal glands observed in the essential hypertensive subjects. Moreover, many studies carried out by other researchers ratified our preliminary findings, and 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 experimentally-induced depression (captivity) was able to provoke a diabetogenic effect in dogs.[10] The fact that these dogs showed greatly increased p-5-HT, and that normalization of the OGTT plus p-5-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

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. Values are expressed as mean ± s.e. (*) p<0.05; (**) p<0.02; (***) p<0.01.*

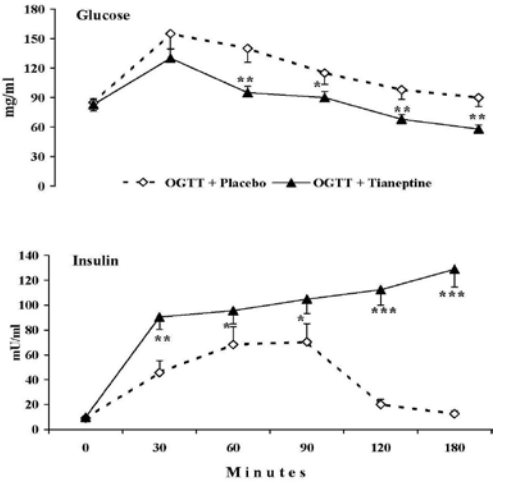
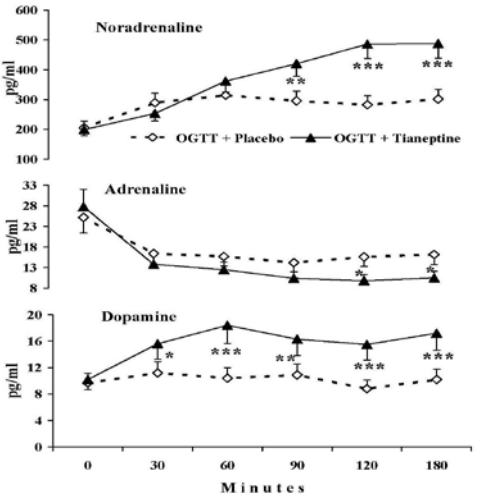


Fig. 2. *The addition of tianeptine to an oral glucose load potentiated the normal NA and DA rises (neural sympathetic activity) as well as the decreases of Ad (adrenal sympathetic activity), always registered troughout this test without the drug. Values are expressed as mean ± s.e. (*) p<0.05; (**) p<0.02; (***) p<0.01.*



beta-cells.[11,12] Furthermore, we also demonstrated that the chronic administration of some dopaminergic (DA) blocking agents, like sulpiride, was able to provoke a diabetogenic effect and a p-5-HT elevation similar to that registered during captivity.[11] Finally, the fact that normalization of clinical, hormonal, and glucose parameters was observed after the interruption of drug administration caused us to postulate that serotonin was the common etiopathogenic factor responsible for both the metabolic and the psychiatric disorder.[13] The above findings were confirmed and published.[9,14,15] Finally, our experiment dealing with the annulment of hyperinsulinism and hypoglycemia by doxepin (a serotonin uptake inhibitor) in a large number of patients[16] led us to explore the possible insulinogenic effect of drugs like tianeptine, which enhances rather than inhibits the uptake of 5-HT.[17]

SUBJECTS AND METHODS

One OGTT plus placebo and one OGTT plus tianeptine test were carried out 2 weeks apart in 38 normal voluntary humans (26 males and 22 females), whose ages ranged from 20 to 63 years (Mean ± SE = 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. In order to disqualify subjects with depression, subjects were rated on a modified Hamilton Depression Rating Scale for Depression and all of them completed the self-rating Beck Depression Inventory.[16] Pregnancy, lactation, smoking and/or alcoholism also excluded subjects.

Analytical Methods

Noradrenaline (NA), adrenaline (Ad), dopamine (DA), plasma free serotonin (f-5-HT), platelet serotonin (p-5-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. A heparinized venous catheter was inserted into a forearm vein at least 30 min prior to the tests. Blood samples were collected at 0, 60, 90, 120 and 180 minutes. Each subject drank a 30% glucose solution (1 g/kg of ideal body weight). 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. Then, blood was promptly centrifuged at 600 rpm for 15 min at 4° C in order to obtain platelet-rich plasma. Two mL of platelet-rich plasma, obtained for determination of platelet serotonin (p-5-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 2 portions for determination of catecholamines and free serotonin (f-5-HT), after which both portions were stored at –70 °C until assayed.

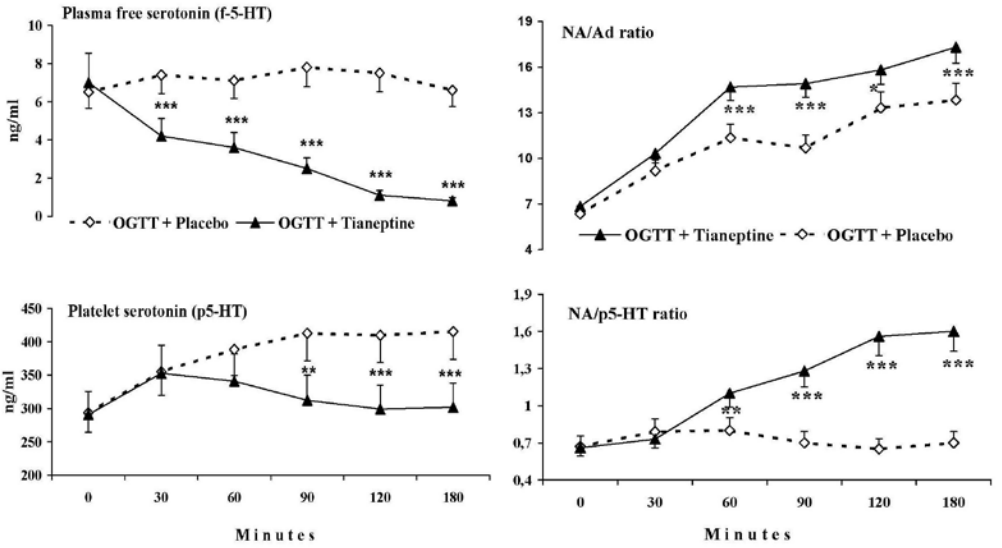
A physician, in constant attendance, noted any symptoms reported by the subjects and monitored heart rate, systolic blood pressure and diastolic blood pressure every 30 minutes.

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,

Fig. 3. *The addition of tianeptine to an oral glucose load reversed the plasma serotonin (f-5-HT) and platelet serotonin (p-5-HT) rises, normally registered troughout this test without the drug. Values are expressed as mean ± s.e. (*) p<0.05; (**) p<0.02; (***) p<0.01.*

Fig. 4. *The addition of tianeptine to an oral glucose load potentiated the normal NA/Ad ratio registered throughout this test without the drug. Evenmore, it provoked the enhancement of the NA/p-5-HT ratio triggered by both the NA rises and the p-5-HT fall. Values are expressed as mean ± s.e. (*) p<0.05; (**) p<0.02; (***) p<0.01.*



S.A. (Caracas, Venezuela). Glass-distilled water was de-ionized and filtered through a Milli-Q reagent grade water system (Millipore, Bedford, MA, U.SA.). 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 µ1 sample loop (Rheodyne; Berodine, Berkeley, CA, U.S.A.). 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 elution with 200 µ1 of 1.0 mo1/1 $HClO_4$ using Regenerated Cellulose microfilters of 0.2 µm pore size (Whatman Inc). We calibrated the instrument with standard plasma: after incubation with acid-washed aluminum

***Table 2**. Correlations (r) found during the OGTT + Tianeptine (T) test.*

	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. Gl	n.s.	n.s.	-.267*	-.338**	-.422**	-.439**
DA vs. Gl	n.s.	n.s.	-.332*	-.365*	-.411**	-.395**
Ad vs. Gl	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NA vs. p-5-HT	n.s.	n.s.	-.244*	-.332*	-.326*	-.299*
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.	-.237*	-.314*	-.333*	n.s.
p-5-HT vs. Gl	n.s.	n.s.	.244*	.338*	.352*	.341*
NA vs. Ins	n.s.	n.s.	.211*	.253*	.324**	.323**
DA vs. Ins	n.s.	n.s.	.199*	.218*	.235*	.239*
Ad vs. Ins	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p-5-HT vs. Ins	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: (*) $p < 0.05$; (**) $p < 0.02$. NA = noradrenaline, Ad = adrenaline; DA = dopamine; p-5-HT = platelet serotonin; HR: heart rate, SBP= systolic blood pressure; DBP = diastolic blood pressure.

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) was added to the plasma pool. Both the standard plasma and the sample plasma were supplemented with 20 µ1 of internal standard (100 ng/ml of dihydroxybenzylamine). The mobile phase was KH2PO4 6.8045 g/L, EDTA 0.100 gm/L, 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 pg/ml, respectively. The intra-assay coefficients of variation were 2.8, 4, and 4 %, 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, U.S.A.), both platelet-rich and platelet-poor plasma were processed in the same way: 200µl of 3.4 mol/L perchloric acid and 50 µ1 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 µ1 was injected into the column. Calibration runs were generated by spiking blank platelet-poor plasma with 50 µ1 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 consisted of 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 an ion-pair agent in a concentration of 4.25 mg/L with a pH of 5.0. The sensitivity of the method for serotonin was 0.1 ng/ml. The intra-assay coefficients of variation for p-5-HT and f-5-HT were 6.2 and 8.7%, respectively.

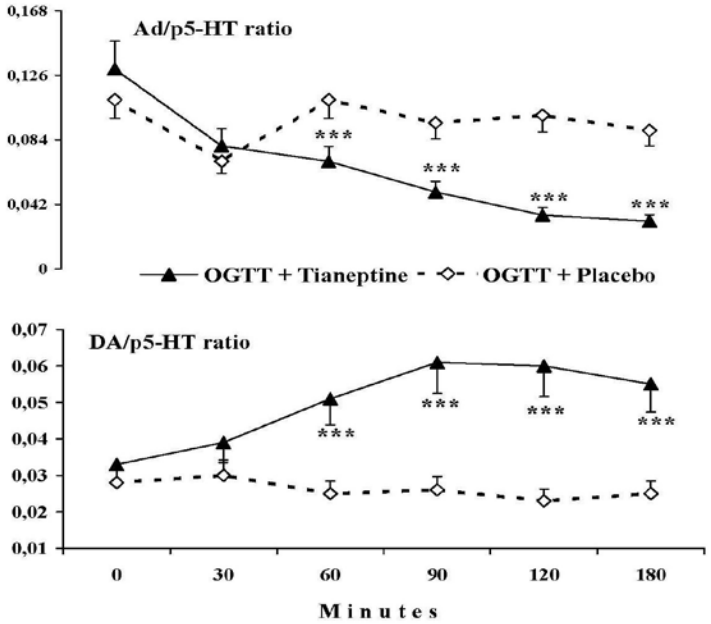
Plasma insulin. This was determined by a radioimmunoassay with an insulin-antibody precipitate.[16]

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 (explorato-

Fig. 5. *The addition of tianeptine to an oral glucose load triggered the fall of both the adrenaline (Ad) and the platelet serotonin (p-5-HT) as well as the Ad/p-5-HT ratio. In addition, significant increases of the DA/p-5-HT ratio were observed. Values are expressed as mean ± s.e. (*) p<0.05; (**) p<0.02; (***) p<0.01.*



ry factor analysis) were used; $p < 0.05$ was considered significant. Dbase Stats (TM) by Ashton Tate and Statview SE and Graphics by Abacus were used for statistical analyses.

RESULTS

Oral Glucose + Placebo Test

The assessment of circulating neurotransmitters after an oral glucose load was carried out according to the present protocol, successfully executed by other, previously published research studies.18 Only NA and p-5-HT circulating values showed significant and sustained rises. These values paralleled insulin increases, and were negatively correlated with 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 observations, both NA and p5HT levels were raised after the oral glucose load, and the NA/p5HT 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 p-5-HT (Fig. 4) were registered. Significant reduction of Ad values was registered throughout the test. No significant changes were observed in DA and f-5-HT. Slight, but significant, decreases in the mean + S.E. values of heart rate and systolic blood pressure (SBP) were registered throughout the test. Significant reductions in the mean values of NA/p-5-HT (Fig. 3), Ad/p-5-HT and DA/p-5-HT ratios were also registered at 60, 90, 120 and 180min (Fig. 5). All of our subjects fell asleep and displayed REM during OGTT, especially during the 90 and 180min intervals. Correlations are in Table 1.

Oral Glucose + Tianeptine Test

Subjects did not show drowsiness throughout the test. A small dose of oral tianeptine added to an oral glucose dose (Fig. 1) triggered insulin secretion associated with changes in the autonomic nervous system. The parameters registered in this study, namely the increase of both NA and DA, and the decrease in Ad (Fig. 2), p-5-HT and f-5-HT circulating levels (Fig. 4) were consistent with an increase in insulin. Consequently, this report will discuss the possible physiological and pharmacological mechanisms underlying the above findings.

Plasma catecholamine changes

Positive correlations were registered between NA and NA/Ad when these parameters were plotted versus DA. This finding strongly suggests that the latter arose from the sympathetic nerves rather than the adrenal glands (Table 2). Furthermore, significant positive correlations were registered between NA and DA versus diastolic blood pressure (DBP), but not versus SBP and heart rate (Table 3). This indicates that neural, not adrenal sympathetic activity was responsible for these changes.

Circulating indoleamine changes

The significant reductions of both p-5-HT and f-5-HT indicate that the drug reversed normal post-glucose increases of these indoleamines (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

A small, but significant rise of glucose levels was registered at the 30min and 60min post-glucose periods. Non-significant increases were found throughout the 90min, 120min, 180min post-glucose periods (Fig. 1).

Plasma 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 4 periods (See Table 2).

DISCUSSION

The insulin secretion triggered by the addition of a small dose of tianeptine to an oral glucose load is associated with the enhancement of neural sympathetic activity. This study observed close positive correlations between NA and DA, and negative correlations between NA and Ad, as well as significant positive correlations between both NA and DA, and insulin. Conversely, significant negative correlations were registered for NA and p-5-HT, and DA and p-5-HT during the post-glucose + tianeptine trial. These findings agree with the progressive and significant reduction of p-5-HT and increases of NA and DA values registered throughout the trial. On the other hand, the maximal fall of both Ad and f-5-HT values interfered with the statistical assessment dealing with these parameters.

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.

The mechanism by which tianeptine, a drug which reduces neural sympathetic activity,17 was able to enhance the normal neurosympathetic drive always registered throughout the OGTT warrants further discussion.

Peripheral mechanisms

Serotonin released from the enterochromaffin cells (ECC) during postprandial parasympathetic drives19,20 modulates both beta and alpha cells.21 It is incorporated into the insulin granules of the former,22,23 exerting 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. This counteracts 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 negate the alpha-cell plus adrenal gland cascade.

Data demonstrates that serotonin loaded into the beta cells localizes to insulin granules, and is additionally co-released with insulin.[26,27] Findings from this study dshow that there is little exocytotic activity of insulin + 5-HT at the basal glucose level, but increasing sugar concentration results in increased insulin release from perfused beta cells. Furthermore, Deeney et al.[26] concluded that serotonin might be considered 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, minimizing the insulin release from the latter. Consequently, the drug would favor this modulatory mechanism.

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 reinforces the predominance of the insulinogenic effect registered in this study. This phenomenon corresponds with other studies, which have found that adrenalectomy interferes with increases in glucagon-induced plasma glucose provoked by intravenous administration of 5-HT.35 Finally, the fact that methysergide, a 5-HT antagonist, can prevent the effects of glucagon provides additional support for the postulation that serotonin, acting at the islet cells directly, is the factor responsible for the above phenomenon.[28,36]

The previous comments are reinforced by others, who have shown that intraperitoneally-injected serotonin increases glucagon and glucose, and reduces insulin plasma

levels. Thus, the negation of these effects, triggered by the addition of tianeptine to the oral glucose load, can be attributed to interference of the drug, not only at CNS, but also at the peripheral level. In addition, adrenodemedullation prevented both the glucose rise and the insulin fall, but not the glucagon rise. This fact indicates that effects at the beta-cell level were mediated by 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 is consistent with the minimization of the parasympathetic drive, which excites the ECC system. This is inferred by the lowering of p-5-HT circulating levels observed throughout the test. Sympathetic nerves bridle the parasympathetic mechanism. It is also important to note that f-5-HT, but not p-5-HT, is able to excite the medullary area postrema (AP), which sends modulatory axons to both vagal and adrenergic medullary nuclei that are 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 f-5-HT. Both circulating Ad and ACh are able to increase f-5-HT; the former because it triggers platelet aggregation, the latter through the interference with the platelet uptake of serotonin.[42]

In short, the negation of both adrenal sympathetic and parasympathetic activities registered in this study results from f-5-HT acting to suppress the stimulatory drives at the area postrema. The area postrema 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] The C1(Ad) nuclei exchanges inhibitory axons with A5(NA) pontomedullary nucleus, which is responsible for neural sympathetic activity.[45,46] Thus, the lack of activity of the former, triggered by tianeptine, minimized the peripheral adrenal sympathetic drive. This favored the absolute predominance of the neural sympathetic activity registered in the present study. 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. The C1(Ad) medullary nuclei responsible for the activity of the adrenal glands 44 receive excitatory 5-HT axons from the dorsal raphe (DR)-5HT nucleus[50,51] and also from the medullary serotonergic nuclei: raphe magnus, raphe obscurus and raphe pallidus.[52] The drug interferes 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 that enhances this mechanism, should eliminate this serotonergic hyperglycemic effect. Consequently, it will interfere with insulin secretion. This postulation correlates with the ability of drugs that interfere with 5-HT uptake (like doxepin, sertraline, paroxetine or fluvoxamine) to counteract hyper-insulinism and hypoglycemia syndrome.[16,53]

In addition, DA plasma rises were also registered in this study. These findings are consistent with preliminary research studies showing that the diabetogenic effect triggered by captivity and/or sulpiride is positively and negatively correlated with 5-HT and DA blood levels, respectively.[11-13] This profile of circulating neurotransmitters contrasts with effects reported in this study. Furthermore, the significant positive correlations of NA and DA shown in the present research indicate that both catecholamines originated 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 cross the blood-brain barrier. They both 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 established data.

In conclusion, both insulin and glucagon cross the blood-brain barrier and excite the C1(Ad) medullary nuclei and the A5(NA), respectively. Both nuclei interchange inhibitory axons.[9,45] Predominance of the former results in neural sympathetic activity plus hyperinsulinism and hypoglycemia. The opposite peripheral profile occurs in response to overactivity of the C1(Ad) nuclei (adrenal sympathetic excitation plus hyperglycemia). This catecholaminergic binomial CNS axis is modulated by the CNS serotonergic axons originating 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. The bulk of quoted evidence demonstrates that while 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 and hyperinsulinism registered in the present study strongly suggests that the drug suppressed the activity of the DR(5-HT) and C1(Ad) axis. This favored the disinhibition of the A5(NA) and the MR(5-HT) binomial.[57] Thus, tianeptine acts on normal glucose-induced neural sympathetic activity in addition to reducing both the parasympathetic and adrenal sympathetic activities. This reinforces the absolute predominance of the action on the neural sympathetic system.

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