Effects of an oral dose of L-glutamic acid on circulating neurotransmitters: Possible roles of the $C_1$(Ad) and the $A_5$(NA) pontomedullary nuclei

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Objective: Investigation of the effects of an oral administration of a small dose of L-glutamic acid on the two peripheral sympathetic branches (neural and adrenal) of the autonomic nervous system.

Research design and methods: Circulating neurotransmitters and cardiovascular parameters were assessed in 28 healthy volunteers before and after the administration of 500 mg of L-glutamic acid or placebo.

Results: The drug triggered a significant and sustained enhancement of the noradrenaline and dopamine circulating levels which were paralleled and positively correlated with the diastolic blood pressure increases. Conversely, both platelet and plasma serotonin showed significant falls throughout the test. Significant positive correlations were registered between noradrenaline, dopamine, and noradrenaline/dopamine ratio versus diastolic blood pressure but not versus systolic blood pressure or heart rate.

Conclusion: The above results allowed us to postulate that the drug provoked a significant enhancement of peripheral neural sympathetic activity and the reduction of adrenal sympathetic and parasympathetic drives. Both sympathetic branches are positively correlated with the $A_5$ noradrenergic and the $C_1$ adrenergic pontomedullary nuclei, which interchange inhibitory axons that act at post-synaptic $\alpha_2$ inhibitory autoreceptors. In addition, we discussed the mechanisms able to explain why the drug acted preferentially at the $A_5$ noradrenergic rather than the $C_1$ adrenergic nuclei.

Keywords: glutamic acid, catecholamines, noradrenaline, serotonin, sympathetic activity

Introduction
The $C_1$ adrenergic (Ad) and the $A_5$ noradrenergic (NA) pontomedullary nuclei are the master controllers of the peripheral adrenal and neural branches of the sympathetic nervous system, respectively. Both central nervous system nuclei interchange inhibitory axons that are crowded by $\alpha_2$ postsynaptic receptors. However, the $C_1$(Ad) but not the $A_5$(NA) neurons are also crowded by imidazole inhibitory receptors. Other evidence showed that adrenal glands secrete adrenaline (80%) and noradrenaline plus dopamine (20%) whereas sympathetic nerves release noradrenaline (80%) plus dopamine (20%), approximately. Both peripheral sympathetic branches may act in association or dissociation in accordance with physiological or pathophysiological demands.1–5

Although it was established that the $C_1$(Ad) nuclei receive excitatory glutamatergic (GLUT) axons which act at N-methyl-D-aspartic acid (NMDA) receptors located at these levels, no definite conclusion has been reached on the direct physiological effect triggered by GLUT axons at the $A_5$(NA) nucleus. Both excitatory and inhibitory blood pressure responses to this interaction have been reported.6–13
In addition to the above, other indirect, polysynaptic mechanisms are able to influence the A_{3}(NA) and C_{1}(Ad) interaction. For instance, the C_{1}(Ad) interchange excitatory axons with the dorsal raphe serotonergic [DR(5HT)]^{14} and the A_{3}(NA) (locus coeruleus)^{15} nuclei whereas the A_{3}(NA) nucleus interchanges inhibitory axons with the A_{3}(NA) neurons and cooperates with the median raphe serotonergic (MR-5HT) and in addition, the A_{3}(NA) receives inhibitory axons from the DR(5-HT) neurons.^{16,17} According to all the above, the C_{1}(Ad) vs A_{3}(NA) direct antagonism is modulated by a complex NA + 5HT pontomedullary circuitry addressed to avoid the black vs white peripheral autonomic nervous system (ANS) responses. In addition to all the above, the peripheral ANS sends signals (neural and/or neurotransmitters) to the C_{1}(Ad) + A_{3}(NA) binomial in order to attenuate the peripheral ANS unbalances triggered by both physiological and pathophysiological circumstances.\(^4\) For instance: electrical excitation of the A_{3}(NA) nucleus provokes blood pressure reduction. This effect is mediated by the cholinergic (ACH) nucleus tractus solitarius which receives excitatory axons from the former.\(^19\) Hence we decided to investigate the effects of an oral dose of L-glutamic acid on circulating neurotransmitters in order to find more information addressed to shed more light on the role played by the A_{3}(NA) neurons on the peripheral sympathetic nervous system.

**Methods**

**Experimental design**

Levels of plasma noradrenaline, adrenaline, dopamine, free serotonin (f5-HT), and platelet serotonin (p5-HT) were measured before (0 minutes) and after (60, 90, 120 minutes) the oral administration of 500 mg of L-glutamic acid in 28 healthy volunteers. Similar measurements were performed two weeks before, in the same volunteers after oral administration of placebo. The group of volunteers comprised 12 men and 16 women, whose ages ranged from 19 to 60 years (mean ± SE = 38.9 ± 7.4). Informed consent was obtained in writing from all volunteers, and the procedure was approved by the ethical committee of FUNDAIME. All volunteers were within 10% of ideal body weight, none had any physical or psychiatric illness. Exclusion criteria included pregnancy, lactation, smoking, and alcohol abuse. Volunteers were recumbent during all procedures. A physician in constant attendance noted any symptoms reported by the subjects and monitored heart rate and blood pressure. The study was conducted in accordance with the guidelines in the Declaration of Helsinki. Written informed consent was obtained after the purpose, nature and potential risks had been explained to the subjects. The experimental protocol was approved by the ethical committee of FUNDAIME.

**Analytical methods**

Noradrenaline, adrenaline, dopamine, plasma-free serotonin, and platelet serotonin were measured throughout the 120-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 fasting. A heparinized venous catheter was inserted into a forearm vein at least 30 minutes before beginning the tests. Blood samples were collected at 0, 30, 60, 90 and 120 minutes. Each subject took an oral dose of 500 mg L-glutamic acid (one tablet) or similar tablet containing placebo. Blood for catecholamines and serotonin assays was transferred to plastic tubes, each containing 20 mg of EDTA plus 10 mg of sodium bisulphite/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 free serotonin (f5-HT), after which the portions were stored at –70°C until assayed.

**Reagents and standards**

Noradrenaline, adrenaline, dopamine, serotonin creatinine sulphate, dihydroxybenzylamine, sodium octyl sulphate, dibutyrylamine, acid-washed aluminium oxide, Na_{2}HPO_{4}, 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 South Africa (Caracas, Venezuela). Acetonitrile and 2-propanol were obtained from Merck South Africa. 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 de-aerated. Standard solutions (1 mmol/L) were prepared in 0.1 mol/L perchloric acid and diluted to the desired concentration.
Equipment
Liquid chromatography was performed using Waters 515 HPLC pump (Waters Corporation, Milford, MA, 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 × 4.6 mm inner diameter Discovery C18 column packed with octadeclisilane 5 µm particles was preceded by a column prefilter of 2 µm porosity, both from Supelco/Sigma-Aldrich. The detection system was a Waters 460 Electrochemical Detector (Waters Corporation). 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 indolamines. The chromatograms were registered and quantified with the Empower software from Waters Corporation. 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 mol/L HClO4 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 noradrenaline, adrenaline and dopamine (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 KH2PO4, 6.8045 g/L, EDTA 0.100 g/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 a pH of 5.6. The flow rate was 0.400 mL/min. The sensitivity of the method 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 indolamines
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: 200 µL of 3.4 mol/L perchloric acid and 50 µL of 5-hydroxy-tryptophan solution (114.5 µg/mL) as internal standard, were added to 1 mL of plasma vortexed and centrifuged at 10,000 rpm for 15 minutes 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 g/L, sodium acetate 4.1015 g/L, EDTA 0.100 g/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 flow rate was 0.610 mL/min. The sensitivity of the method 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.

Statistical methods
Results are presented as the mean ± SEM. Multivariate analysis of variance (ANOVA) with repeated measurements, paired t test, and correlation coefficients (exploratory factor analysis) were used. Dbase Stats™ (Ashton Tate, Torrance, CA, USA) and StatView SE + Graphics (SAS Institute, Cary, NC, USA) were used for statistical analyses.

Results
Noradrenaline was significantly raised at all periods following L-glutamic acid administration. Maximal increases in plasma noradrenaline occurred at the 120 minute period. Sustained, progressive and significant decreases of adrenaline values were observed throughout the test. Dopamine plasma levels increased significantly. The NA/Ad ratio showed greatly significant and progressive increases as of the first 60-minute period (Figure 1). We noted significant positive correlations between noradrenaline and dopamine values between the 60- and 120-minute periods.

Moderated but significant decreases of free serotonin in the plasma and platelet serotonin were found throughout the 120 minutes of the experimental trial, the f-5HT/p-5HT ratio did show significant and progressive reduction from the 60 min period until the end of the trial (Figure 2).

Neither heart rate nor systolic blood pressure showed significant changes throughout the experimental trial. Diastolic blood pressure showed slight but significant increase from the 60-minute period until the 120-minute period (Figure 3).
Significant positive correlations were found between NA vs NA/Ad ratio ($r = 0.83$; $P < 0.005$) and NA vs DA ($r = 0.61$; $P < 0.01$). The former tended to rise from the first to the last (post L-glutamic acid) period. The NA vs DA correlation showed progressive rises throughout the trial. These findings indicate that both NA and DA arose from the same source (sympathetic nerves) rather than adrenal glands. The parallelism registered between these two parameters is consistent with the NA vs DA positive correlation.

Noradrenaline vs diastolic blood pressure correlation values at post drug periods were: $r = 0.66$, 0.78, and 0.85 ($P < 0.01$; $P < 0.01$; and $P < 0.005$, respectively). Dopamine vs diastolic blood pressure-positive correlations were also significant at 90-minute and 120-minute periods; $r$ values were: 0.63 and 0.78, respectively ($P < 0.01$).

Significant positive correlations were found between DA vs diastolic blood pressure, at the 90-minute and 120-minute periods; $r$ values: 0.68 and 0.72 ($P < 0.02$ in both cases).

Significant and progressive negative correlations were found between the NA/Ad ratio vs the f5-HT/p5-HT ratio from the 60-minute period until the trial’s end; $r$: $-0.65$, $-0.72$, and $-0.84$ ($P < 0.02$; $P < 0.02$; and $P < 0.01$, respectively).
Significant positive correlations were found between NA/Ad vs diastolic blood pressure values at 90-minute and 120-minute periods; r values: 0.81 and 0.96, respectively (P < 0.005 in both cases).

Discussion
The results obtained from the present study demonstrated that a small oral dose of glutamate was enough to enhance neural sympathetic activity, as demonstrated by the increase of the NA/Ad plasma ratio plus the DBP rises.\(^{3,4,16,17}\) In addition, the minimization of the plasma-free serotonin (5-HT) (in the absence of platelet aggregation) should be explained by the decrease of acetylcholine plasma levels (parasympathetic hypooactivity), which competes with serotonin for platelet uptake.\(^{19,20}\) Furthermore, the significant fall of total blood serotonin [platelet serotonin (p5-HT + f5-HT)] registered in this study should be attributed to the attenuation of the intestinal (enterochromaffin cells) secretion which depends on the excitatory parasympathetic plus the inhibitory sympathetic nerves.\(^{21-23}\) Taking into account that GLUT axons excite both the C\(_1\) (Ad) medullary nuclei and the A\(_3\) (NA) neurons, which are crowded by NMDA receptors,\(^ {24,25}\) the facts sprouted from this study are consistent with the postulation that the drug increased A\(_3\) (NA) rather than the former nuclei.

The A\(_3\) (NA) and the C\(_1\) (Ad) nuclei present physiological differences when compared to the A\(_3\) (NA) nucleus. For instance, the two former should receive excitatory drives to fire (clonic excitation),\(^ {26}\) whereas the A\(_3\) (NA) neurons shows tonic, continuous firing activity.\(^ {18}\) This latter displays minimal firing at the REM sleep period, at which level, very low diastolic blood pressure is registered.\(^ {27,28}\) In addition the two former nuclei [A\(_3\) (NA) and C\(_1\) (Ad)] but not the A\(_3\) (NA) are excited by ACh-axons which arise from the medullary vagal complex and the pedunculopontine nucleus (PPN).\(^ {29}\) However, not only the A\(_3\) (NA) and the C\(_1\) (Ad) but also the A\(_3\) (NA) nucleus send modulatory axons to the medullary acetylcholinergic complex nucleus tractus solitarius, nucleus ambiguus, nucleus reticularis giganto cellularis and others.\(^ {30,31}\)

Findings obtained from the present study showed that a small oral dose of glutamate was able to enhance neural sympathetic but not adrenal sympathetic activity. These findings indicated that the A\(_3\) (NA) but neither the C\(_1\) (Ad) nor the A\(_3\) (NA) was excited by the drug. These results fit well with the above mentioned findings referred to the tonic but not clonic activity display by the former.\(^ {32-36}\)

In addition to all the above, other mechanisms should be commented. For instance, both the A\(_3\) (NA) and the C\(_1\) (Ad) nuclei send inhibitory and excitatory axons, respectively, to the medullary vagal complex which are responsible for the blood pressure and the heart rate (HR) modulation thus, results obtained from this research fit well with the A\(_3\) (NA) but not the C\(_1\) (Ad) excitation. Additionally, this postulation is in accordance with other studies which showed that the C\(_1\) (Ad) but not the A\(_3\) (NA) are crowded by excitatory ACh receptors\(^2\) which would interfere with both the DBP and plasma NA rises, registered in this study.

Some other experimental studies reported that direct administration of glutamic acid into the A\(_3\) (NA) nucleus provoked blood pressure reduction instead of rise.\(^ {18}\) With respect to this, we believe that this should be attributed to the enhancement of the inhibition of the C\(_1\) (Ad) plus the excitation of the vagal medullary nuclei (nucleus tractus solitarius, dorsal vagal complex). Both adrenergic and parasympathetic nuclei receive inhibitory and excitatory axons from the A\(_3\) (NA) nucleus. Furthermore, considering that the C\(_1\) (Ad) rostral ventrolateral medullary nuclei is innervated by excitatory glutamategic axons which act at NMDA receptors, located at this level, the minimization instead of the enhancement of both plasma Ad and cardiovascular parameters (systolic blood pressure and HR) registered in this study, discards the above possibility.

Glutamate axons are also able to excite NA neurons located at the A\(_3\) (NA) (locus coeruleus) neurons which are crowded by NMDA receptors, however, these NA neurons exerts modulatory but not direct effects at the peripheral sympathetic activity. A\(_3\) (NA) axons are able to inhibit and excite A\(_3\) (NA) and dorsal vagal complex, respectively, which attenuate excessive blood pressure rises and falls, respectively.\(^ {37}\) According to all the above, it is possible to postulate that the NA/Ad plasma ratio plus the diastolic blood pressure rises triggered by a small oral dose of L-glutamic acid administered to normal subjects excited neural but not adrenal sympathetic activity.

Additional findings which show that the A\(_3\) (NA) and the C\(_1\) (Ad) but not the A\(_3\) (NA) receive ACh excitatory and GABA inhibitory axons\(^ {38-46}\) offer more data which helps to understand the discriminative effects exerted by the oral glutamate challenge.

Considering that GLUT axons exert powerful excitatory effects on the MR serotonergic (5-HT) but not at the DR(5-HT) neurons,\(^ {14,17,47}\) we should include some additional comments to this respect. The DR(5-HT) interchanges excitatory axons with the C\(_1\) (Ad) nuclei.\(^ {14,40}\) Conversely, the A\(_3\) (NA) receives inhibitory axons from the DR(5-HT) nucleus.\(^ {48}\) In addition, the MR(5-HT) neurons, which display clear antagonism
with the DR(5-HT) nucleus, cooperate with the A₅(NA) drives at the nucleus centralis amygdala (CEA) and the bed nucleus stria terminalis levels. This additional central nervous system circuitry, which includes hypothalamic circuitry, is responsible for the peripheral neural sympathetic activity, whose hyperactivation is able to modulate the C₅(Ad) + DR(5-HT) axis, responsible for the peripheral adrenal sympathetic branch, as demonstrated in the present experimental study.

The results obtained from this study, helps to understand the controversial findings reported by Neil and colleagues, which showed that the direct glutamate injection at the A5(NA) nucleus was able to annul rather than excite neural sympathetic activity. This A₅(NA) neural sympathetic activation reported in this study is able to antagonize the DR(5-HT) plus C₅(Ad) axis responsible for the peripheral adrenal sympathetic branch. The fact that GLUT axons excite both the A₅(NA) plus the MR(5-HT) neurons but not the DR(5-HT) nucleus (which is crowded by inhibitory GABA neurons), fits well with all the above.

Summarizing, the oral administration of a small dose of glutamate (500 mg) was able to enhance A₅(NA) neurons which display tonic rather than clonic firing activity but not the C₅(Ad) plus A₅(NA) neurons that display clonic firing activity. These findings fit well with others showing that the two latter but not the former receive ACh excitatory and GABA inhibitory axons. Finally, considering that A₅(NA) neurons do not cease to fire might ( tonic activity) explain its accessibility to the GLUT agonist which is not counteracted by inhibitory GABA drives.

**Disclosures**

The authors report no conflicts of interest in this work.

**References**


