Effects of Desipramine on Circulating Neurotransmitters in Patients Affected by Neural Sympathetic or Adrenal Sympathetic Hyperactivity

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ABSTRACT

The circulating neurotransmitters noradrenaline, adrenaline, dopamine, platelet serotonin, plasma serotonin, and tryptophan were assessed before and after a desipramine challenge (10 mg injected intramuscularly) in patients affected by neural sympathetic predominance (N), adrenal sympathetic predominance (A), and normal controls (C). Maximal increases of the noradrenaline/adrenaline ratio were found in type N patients, whereas minimal but significant increases were registered in type A patients. In addition, statistical data showed that dopamine arose from sympathetic nerves in type N subjects and from the adrenal glands in type A patients. Successful neuropharmacological therapy guided by the above neurotransmitter profiles were able to normalize both the clinical syndromes plus the autonomic nervous system disorders. With respect to this, we present the results obtained in 154 type N and 110 type A patients treated and carefully controlled throughout long time periods. According to all the above, we postulate the existence of 2 types of autonomic disorders that underlie diseases and, in addition, we found that type N and type A diseases are frequently associated with the TH-1 and TH-2 immunological profiles, respectively.

INTRODUCTION

We have assessed the peripheral autonomic nervous system (ANS) during the last 50 years in both mammals and humans by measuring different types of physiological parameters (gastrointestinal, cardiovascular, hormonal, metabolic, and circulating neurotransmitters). In

addition to the above, considering that we have assessed all circulating neurotransmitters in patients affected by a great deal of psychological and somatic diseases both before and after the recovery periods, we are obliged to include a great bulk of our published research studies in order to support the rationality of the present summarized report. The assessment of circulating neurotransmitters has been performed during both sleep[1-3] and wake periods,[4] as well as before and after different types of stress challenges (orthostasis and exercise,[5,6] oral glucose load[7,8]) and also before and after different types of drugs that act at both the peripheral nervous system and central nervous system (CNS) level.[9-16] In addition, we have attempted many types of neuropharmacological therapies, guided by the results sprouted from the above mentioned research work. Furthermore, information obtained from this research work allowed us to find the CNS + the peripheral ANS profile that underlies psychological disorders: schizophrenia,[17-22] endogenous depression[23,24]; endocrinological disorders: hyperinsulinism,[14,25-27] infertility[28]; gastrointestinal disorders: duodenal ulcer, gastritis,[29,30] pancreatitis,[31-34] biliary dyskinesia,[35-41] irritable bowel syndrome,[41-48] ulcerative colitis,[49] Crohn's diseases,[50,51] cystic fibrosis,[52] carcinoid tumor[53,54]; cardiovascular disorders[55-58]; essential and non-essential hypertension[7,15,16,59-61]; vascular thrombosis[31,55,62-64]; Raynaud's disease[65]; hematological disorders: thrombocytopenic purpura,[66] polycythemia vera[67]; neurological diseases: myasthenia gravis,[68] trigeminal neuralgia,[69] multiple sclerosis and Guillian Barre[65]; rheumatological diseases: rheumatoid arthritis, fibromyalgia, scleroderma[65]; malignant diseases[50,70-78]; and respiratory disorders: pulmonary hypertension, bronchopulmonary fibrosis,[42,55-57] and bronchial asthma.[79-85]

In addition to the above, we published many research articles that allow us to understand the solid connections that exist between the CNS disorders underlying the different types of stress and depression with the clinical syndromes that, indeed, are only peripheral facades that express the CNS disorders that travel throughout the distinct outlet ANS-ducts.[4,6,24,42,43,74,86-88] According to all the above, we will present evidence showing that the infinite number of facades (diseases) converge to the CNS, at which level depend on a limited number of neurophysiological disorders that can be successfully treated throughout adequate neuropharmacological manipulations. The above evidence sprouted from findings showing that adrenal sympathetic or neural sympathetic predominance underlies most diseases. The former group presents with a low NA/Ad plasma ratio whereas the latter depends on the opposite profile (very high NA/Ad plasma ratio).

In the present study we will report the effects provoked by desipramine, a selective noradrenaline-uptake inhibitor, on circulating neurotransmitters plus neuroautonomic parameters, in both normal and diseased subjects. The fact that the intramuscularly (IM) administered drug is not taken up by the liver (as occurs after the oral administration) should provoke more direct and specific effects on CNS noradrenergic (NA) neurons, quickly potentiating them. In addition, the drug will act at those NA terminals that are releasing NA but not at the terminals of the NA hypoactive neurons. Thus, the assessment of the peripheral ANS effects, triggered by the drug, should help to predict which noradrenergic nucleus is active in the patient (A6 or A5). With respect to this, it has been definitively established that neural sympathetic activity (NA released from sympathetic nerves) depends on the excitation of the A5(NA) pon-

tomedullary nucleus [which interchanges inhibitory axons with the pontine A6(NA) and the medullary C1(Ad) nuclei].[89] These latter nuclei are responsible for the adrenal glands secretion (80% of adrenaline).[90-93] According to the above, the desipramine challenge will increase NA/Ad ratio in subjects affected by neural sympathetic overactivity; conversely, the drug would not provoke such neural sympathetic excitation in patients affected by the adrenal sympathetic overactivity, because their A5(NA) neurons are silenced by both the Ad released from the C1(Ad) and the NA released from the hyperactive A6(NA) axons.[94-98] At the peripheral level, we would not find the same neural sympathetic response registered in the other 2 groups, thus, this test would allow a clear distinction between the 2 pathophysiological syndromes.

SUBJECTS AND METHODS

This study included 264 diseased subjects and their age- and sex-paired normal controls. We separated 2 groups of patients according to their neuroautonomic profile: Group A (adrenal sympathetic predominance) and Group N (neural sympathetic predominance). Group A showed a noradrenaline (NA) over adrenaline (Ad) (NA/Ad) plasma ratio ≤5, whereas group N included a NA/Ad ratio ≥10. According to this criteria, we quoted 110 group A patients: 5 reflux esophagitis, 7 duodenal ulcer, 7 carcinoid syndrome, 9 irritable bowel syndrome-diarrheic period, 7 ulcerative colitis, 6 acute pancreatitis (after remission), 12 non-essential hypertension, 3 paroxysmal supraventricular tachycardia, 3 atopic dermatitis, 5 dengue, 5 influenza, 5 chronic hepatitis C, 5 HIV infection, and 31 malignant diseases relapsing period (1 esophageal carcinoma, 4 gastric adenocarcinoma, 2 colon adenocarcinoma, 3 malt lymphoma, 5 no Hodgkin lymphoma, 1 Antoni type B schwannoma, 6 mammary adenocarcinoma, 2 bladder adenocarcinoma, 2 pulmonary adenocarcinoma, 3 melanoma, 2 nephroma); and 154 group N patients: 4 autoimmune gastritis, 9 irritable bowel syndrome–spastic period, 5 biliary dyskinesia (no gallbladder emptying), 3 cystic fibrosis, 2 pancreatic cyst, 3 pancreatic cancer, 3 Crohn's disease, 7 essential hypertension, 6 vascular thrombosis, 7 mammary plus ovary cysts, 2 Hashimoto's thyroiditis, 6 Sjögren syndrome, 4 type I diabetes mellitus, 8 hyperinsulinism, 4 rheumatoid arthritis, 2 primary sclerosis cholangitis, 4 fibromyalgia, 2 aplastic anemia, 5 thrombocytopenic purpura, 4 polycythemia vera, 13 myasthenia gravis, 5 multiple sclerosis, 9 bronchial asthma (non acute periods), 5 scleroderma, 3 pemphigus, 3 carpal tunnel syndrome, 9 endogenous depression, 7 schizophrenia, 3 post-traumatic stress disorder, and 7 attention-deficit hyperactive disorder. With respect to this, it should be taken into account that type A patients (which show highest levels of adrenaline plus cortisol in plasma >22 μg/mL at morning periods) are considered as uncoping-stressed patients (severely ill).[86] Conversely, type N patients (cortisol plasma levels <12.5 Ìg/mL) are affected by chronic diseases that present few or none remission periods. This type of patient usually presents with a longer survival time than the former.

We measured levels of plasma noradrenaline (NA), adrenaline (Ad), dopamine (DA), free-serotonin (f-5HT), tryptophan (TRP), and platelet-serotonin (p-5HT) before (-30 min and 0 min) and after (30 min, 60 min, 90 min, and 120 min) the IM administration of 10 mg of desipramine in both patients and controls (we found that it is the minimal dose able to provoke significant changes of all circulating neurotransmitters in normal subjects). This study has been approved by the ethical committee

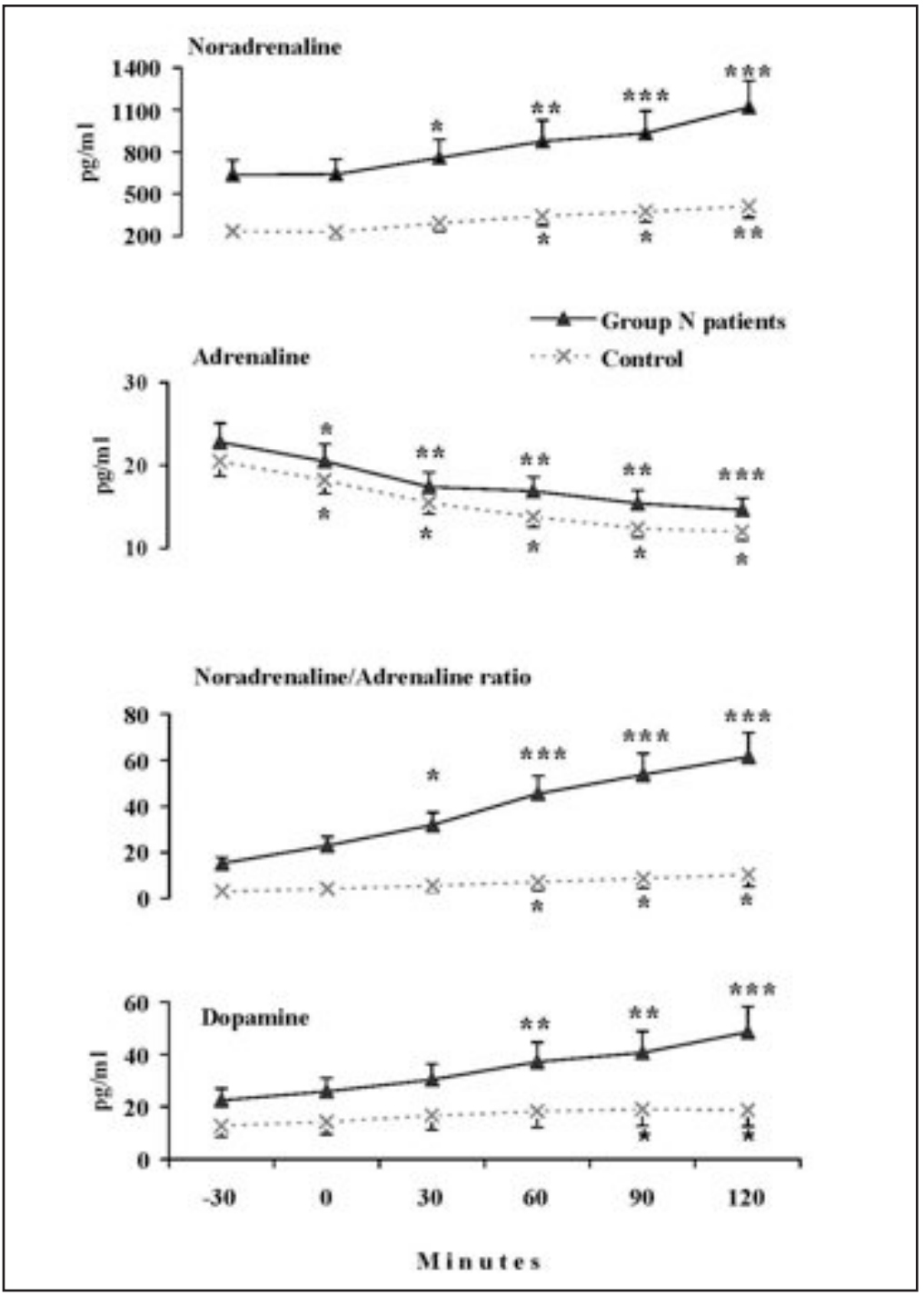

Figure 1. Noradrenaline (NA), adrenaline (Ad), NA/Ad plasma ratio, and dopamine (DA) before and after the IM injection of 10 mg of desipramine. The 2 groups: N (n = 154) = neural sympathetic predominance and their matched controls showed significant increases of NA, NA/Ad, and DA values. The above increases were more significant in N than in control subjects. Both groups showed significant reductions of Ad plasma values. Significance (*) indicates comparison between pre-drug vs post-drug periods. Values are expressed as mean ± SE.

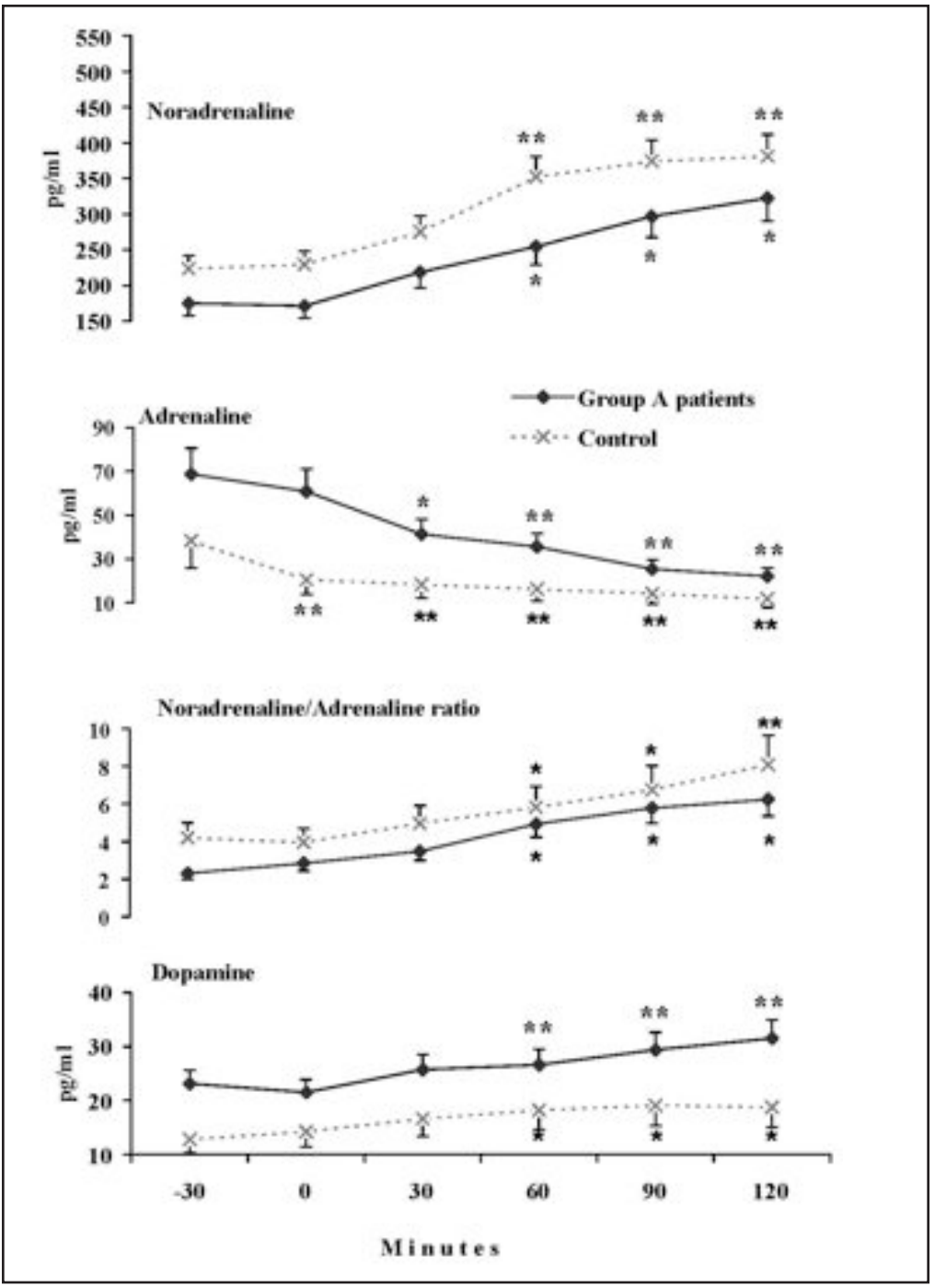

Figure 2. Noradrenaline (NA), adrenaline (Ad), NA/Ad plasma ratio, and dopamine (DA) before and after the IM injection of 10 mg of desipramine in 110 subjects affected by adrenal sympathetic predominance (type A patients) and their matched controls. The NA + NA/Ad + DA rises triggered by the drug were significantly greater in type A patients than in their control subjects. Conversely, desipramine provoked significant reductions of Ad in both type A patients and control subjects. Significance (*) indicates comparison between pre-drug vs post-drug periods. Values are expressed as mean ± SE.

of FUNDAIME and has been performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki. Written informed consent was obtained from all volunteers and they were within 10% of ideal body weight, none had any physical or psychiatric illness. Exclusion criteria included pregnancy, lactation, smoking, and alcohol abuse. Subjects were recumbent during all procedures. A heparinized venous catheter was inserted into a forearm vein at least 30 min before the test. We used cold, plastic syringes to collect blood samples at the times specified above. Desipramine 10 mg IM was administered after the second blood sample (0 min) was obtained. Blood samples were obtained for measuring plasma neurotransmitters and platelet aggregation. Blood for measuring plasma neurotransmitters was transferred to plastic tubes, each containing 1 mL of an anti-oxidant solution (20 mg of EDTA plus 10 mg of sodium metabisulphite/mL). The tubes were carefully inverted several times and placed on ice until centrifugation. To obtain platelet-rich plasma (PRP), we centrifuged the tubes at 600 rpm at 4°C for 15 minutes. We stored 2 mL of PRP at -70°C until needed for determination of p-5HT levels. The remaining blood was centrifuged again at 7,000 rpm. We stored 2 aliquots of the supernatant, which was platelet-poor plasma (PPP), at -70°C until needed for assays of catecholamine and f-5HT. Blood samples for platelet aggregation were processed immediately. A physician in constant attendance monitored heart rate (HR) and blood pressure, and noted any symptoms reported by subjects.

Analytical Assays

Neurochemistry

Plasma catecholamine and serotonin samples were measured in duplicate, and all determinations were made at the same time. We used reverse phase, ion pair high-pressure liquid chromatography with electrochemical detection.[99-101] Optimization of chromatographic conditions allowed us maximal sensitivity and reproducibility.

Reagents and Standards

Noradrenaline, adrenaline, dopamine, serotonin creatinine sulfate, dihydroxybenzylamine, 5-hydroxy-tryptophane, sodium octyl sulfate, dibutylamine KH_2PO_4, citric acid, sodium acetate, acid-washed aluminum oxide, desipramine, and EDTA were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Microfilters were purchased from Bioanalytical Systems Inc. (West Lafayette, IN, USA). Acetonitrile and 2-propanol were obtained from Riedel-de-Haen AG (Frankfurt, Germany). Glass-distilled water was deionized and filtered through a Milli-O reagent grade water system (Millipore, Bedford, MA, USA). Solutions and solvent were filtered through a 0.2 µm Millipore filter and were vacuum deaereated. 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 pumps (Waters Co., Milford, MA, USA) equipped with 7125i Rheodyne valve injector fitted with a 50-µL sample loop for detection of catecholamines, and 100-µL sample loop for p-5HT and f-5HT detection (Rheodyne, Berodine, Berkeley, CA, USA). For catecholamine assays, a 15 cm × 4 mm ID Discovery™ analytical column packed with C18 3-µm particles was used, fitted with a precolumn filter 0.2 µ (Sigma-Aldrich Co., St. Louis, MO, USA).

The detection system was a Waters 460 Electrochemical Detector (Waters Co., Milford, MA, USA). A potential of 0.70 volts was applied to the working

electrode (glassy carbon) vs the Ag-AgCl reference electrode. The chromatograms were registered and quantified using Millennium software (Waters Co., Milford, MA. USA).

Catecholamine Assay

These were performed by extraction onto 20 mg of acid-washed alumina followed by their elution with 200 µL of 0.2 mol/L $HClO_4$ using BAS (Bioanalytical Systems) microfilters. The instrument was calibrated with standard plasma. After incubation with acid-washed aluminum oxide, a plasma pool free of catecholamines was obtained. This was processed similarly to plasma samples, but 20 µL of standard solution containing noradrenaline, adrenaline, and dopamine (50 ng/mL each) was added to 1 mL of the plasma pool to obtain the standard plasma. Both standard plasma and sample plasma were supplemented with 20 µL of internal standard solution (dihydroxybenzylamine 100 ng/mL). The mobile phase was composed of KH_2PO_4 50 mmol/L, EDTA 25.16 nmol/L, sodium octyl sulfate 2.37 mmol/L, di-N-butylamine 100 µL/L, and acetonitrile 2.5% (v/v) with pH adjusted to 5.2. Catecholamine determinations were performed after injection of 50 µL of processed plasma. Correction for dilution was performed. Concentrations are expressed in pg/ml. The sensitivity of this method for noradrenaline was 3.2 pg/mL, for adrenaline was 4.2 and for dopamine it was 2.5 pg/mL. The intra-assay coefficients of variation were 2.3%, 3.6%, and 2.3% for noradrenaline, adrenaline, and dopamine, respectively. The inter-assay coefficients of variation were 2.6%, 3.9%, and 3.8%, respectively.

Serotonin Assay

After sonication of PRP to disrupt any intact platelets (Ultrasonic Liquid Processor, model 385, Heat Systems Ultrasonic, Inc., Farmingdale, NY, USA), both PRP and PPP were processed in the same way: 200 µL of 3.4M perchloric acid as deproteinizing agent and 10 µL of 5-OH-tryptophane solution (80 µg/mL) as internal standard, were added to 1 mL of plasma, vortexed and centrifuged at 10,000 rpm × 15 min at 4°C. The clear supernatant was filtered through a 0.22 µm membrane (Millipore) and injected in the HPLC. Calibration runs were generated by spiking plasma blank containing 50 µL of 5HT solution (10 µg/mL) and 10 µL of 5-OH-tryptophane solution (80 µg/mL). This standard plasma was processed in the same manner as samples. PRP serotonin (p-5HT) and PPP serotonin (f-5HT) levels were determined after injection of 100 µL of the deproteinized sample onto a 30 cm × 4.0 mm Discovery™ column filled with C18 5 µm. The mobile phase was composed of citric acid 20 mol, sodium acetate 50 mol, sodium octyl sulfate 6.45 nmol, dibutylamine 100 µL/L, propanol 3.5% (v/v); pH was adjusted to 4.9, flow rate 0.70 mL/min. The sensitivity of this method for plasma serotonin was 0.18 ng/mL intra-assay coefficients of variation were 2.8% for platelet-rich plasma serotonin and 3.1% for platelet-poor plasma serotonin, respectively. Inter-assay coefficients of variation were 3.5% and 5.2%, respectively. Correction factor for dilution was used. Concentrations are expressed in ng/mL. Platelet serotonin value = PRP serotonin value (total circulating serotonin) minus PPP serotonin value (f-5HT).

Platelet Aggregation

Blood was collected with citrate-phosphate dextrose (1:9 v/v) as the anticoagulant. Blood was subsequently centrifuged at 120 × g for 10 minutes to prepare PRP. Aggregation studies were carried out according to Born's method,[102] and aggregation was induced by ADP and collagen at final concentra-

tions of 4 μmol/L and 4 μg/mL, respectively. Maximal aggregation, expressed as the percentage of maximal light transmission, was measured.

Statistical Analyses

Results are expressed as mean ± SE. Multivariate analyses of variance with repeated measurements, paired *t*-test, and correlation coefficients (exploratory factor analysis) were employed in interpreting the data yielded by this investigation. Differences were considered significant at $P < 0.02$. Dbase Stats™ by Ashton Tate and SE by Abacus were used for statistical analyses and Excel for graphics.

RESULTS

Normal Subjects

Catecholamines

The results obtained from the present study demonstrated that a small dose of desipramine (10 mg IM injected) triggers a small but significant increase of the peripheral neural sympathetic activity as inferred from the significant increase of the NA/Ad plasma ratio. A small but significant increase of DA paralleled the NA increase.

Indolamines

Although platelet serotonin (p-5HT) showed non-significant changes throughout the 120 minutes of the experimental trial, the f-5HT/p-5HT ratio we found to be normal at both pre-drug periods but showed a sudden fall at the post-drug periods from the first 30-min period until the end of the trial.

Plasma level of tryptophan did not show significant changes throughout the experimental study.

Correlations

Significant negative correlations were found between NA/Ad ratio vs DA at 90 min and 120 min (r: -081, and -0.86, respectively; $P < 0.001$ in both cases). These findings indicate that both NA and DA arose from the same source (sympathetic nerves) rather than adrenal glands.

Noradrenaline vs diastolic blood pressure (DBP) correlation values at post-drug periods were: 0.61, 0.69, 0.75, 0.79; $P < 0.01$ in all cases. Significant negative correlations were found between DA and DBP at the 2 last periods (r: -0.53, -0.59; $P < 0.01$ at both periods).

Significant negative correlations were found between the NA/Ad ratio vs DA plasma values at the 90-min and 120-min periods ($P < 0.02$ at both periods).

Neither HR nor systolic blood pressure (SBP) showed significant changes throughout this trial, however, DBP showed slight but significant increase from the 60-min period until 120-min period. Significant positive correlations were found between NA/Ad and DBP values at those periods (r: 0.66, 0.70, and 0.75; $P < 0.01$ in all cases).

Significant and progressive negative correlations were also found between the NA/Ad ratio and the f-5HT/p-5HT ratio at the 2 last periods (-079, and -0.84; $P < 0.01$ and $P < 0.005$). No significant correlation was found between f-5HT, p-5HT, and tryptophan values.

Patients

Group N (Neural Sympathetic Predominance)

The results obtained in the present study demonstrated that the IM administration of despiramine provoked an enhancement of the neural sympathetic activity (NA/Ad ratio) of type N patients (Figure 1). Significant increases of DBP and decreases of the HR paralleled neural sympathetic enhancement. Significant positive correlations were registered between the DBP vs NA/Ad rises (Group N = r: 0.68, 0.73, 0.78, and 0.81; $P < 0.01$ in all cases). In addition,

significant negative correlations were registered between DBP rises and DA increases at the same post-drug period (r: -0.65, -0.72, -0.83, and -0.86; $P < 0.01$ in all cases). Finally, significant positive correlations were registered between NA/Ad vs DA rises (r: 0.65, 0.72, 0.75, and 0.81; $P < 0.01$ in all cases) as well as between NA vs NA/Ad (r: 0.62, 0.65, 0.69, and 0.72; $P < 0.001$).

Summarizing the above results, it seems obvious that desipramine triggered additional enhancement of the neural sympathetic overactivity registered in these patients before the drug administration. These drug-induced neuroautonomic effects were paralleled by the acute clinical worsening of the type N patients.

Group A (Adrenal Sympathetic Predominance)

These patients presented lowered NA plus lowered NA/Ad plasma ratio plus increased of both SBP/DBP ratio and HR. These parameters' disorders were attenuated after the desipramine administration (Figure 2). Although DA plasma values were also increased by the administration of the drug, no DBP change was registered in these patients as that reported in patients affected by neural sympathetic predominance.

Increased platelet aggregability was registered in all these patients. Normalization of this disorder was obtained after the desipramine administration

Correlations

NA/Ad vs DBP correlation values at the last 3 post-drug periods were 0.62, 0.69, and 0.76 ($P < 0.01$ in all cases). Ad vs DA positive correlations were registered at all post-drug periods (0.62, 0.65, 067, 071; $P < 0.002$, $P < 0.01$, $P < 0.01$, and $P < 0.01$). No significant correlation was registered between DA vs DBP. Although f-5HT showed abrupt fall since the first post-drug period, no significant correlations were registered between f-5HT, p-5HT, and f-5HT/p-5HT ratio when tested vs SBP, DBP, or HR.

DISCUSSION

Normal Subjects

The results obtained from the present study demonstrated that a small dose of desipramine (IM injected) triggers a small but significant increase of the peripheral neural sympathetic activity, as inferred from the significant increase of the noradrenaline (NA) over adrenaline (Ad) (NA/Ad) plasma ratio. The small but significant increase of DA should also be attributed to the excitation of the DA pool located at the sympathetic nerves.[103-105] This DA is co-released with NA during the excitation of neural sympathetic activity, as inferred from the close significant negative correlation between the NA/Ad ratio vs DA at the 90-min and 120-min periods ($P < 0.001$ in both cases).

The abrupt fall of the f-5HT levels triggered by the drug should be associated to the well-known anti-ACh activity effect that it provokes.[23] This phenomenon would depend on the abrupt reduction of the ACh-plasma level provoked by desipramine.[106,107] The DBP rises registered at the 60-min, 90-min and 120-min post-drug periods should be also attributed to the enhancement of the neural sympathetic activity triggered by the drug, as inferred from the significant positive correlations registered between the NA/Ad ratio vs DBP at the last 2 periods. Finally, the fact that we registered significant negative correlations between DBP and DA plasma values at these 2 last periods support the postulation that this neurotransmitter arose from the sympathetic nerves, at which level a DA pool exists that modulates the release of NA from these nerves by acting at presynaptic inhibitory D2

receptors.[104,108] Finally, the facts showing that neither SBP nor HR changes were registered during the post-drug periods are in accordance with the absence of significant Ad oscillations throughout the desipramine challenge. This presumption receives additional support from the demonstration that the NA/Ad ratio showed significant negative correlations when plotted versus DA plasma values at the 90-min and 120-min periods. Summarizing, the facts showing that NA/Ad values were positively correlated with DBP values support the postulation that neural but not adrenal sympathetic activity was responsible for the increase of both parameters. Furthermore, the significant negative correlations registered between the NA/Ad vs the f5HT/p5HT ratios are consistent with the postulation that the excitation of the neural sympathetic activity triggered by the drug was responsible for the inhibition of the parasympathetic activity.

Group N (Patients Showing Neural Sympathetic Predominance)

These patients are characterized by the highest levels of plasma NA + DA plus raised NA/Ad ratio values. These findings allow us to postulate that they are affected by frank neural sympathetic over adrenal sympathetic predominance.[60] The fact that desipramine exacerbated the raised NA/Ad ratio + the DA pre-drug plasma values registered in these patients strongly supports the postulation that these overflow of circulating NA + DA but not Ad arose from the hyperactive sympathetic nerves rather than from the adrenal glands.[7,109-112]

Taking into account the ability of a small dose of desipramine to provoke the dramatic changes reported in this study seems obvious to postulate that such effects are CNS-induced rather than through peripheral mechanisms. With respect to this, it should be remembered that parentally injected desipramine was not retained by the liver and quickly crosses the blood brain barrier. This drug (a NA-uptake inhibitor) would act at the more active rather than at hypoactive NA neurons, which are not releasing NA. Thus, considering that neural sympathetic activity is closely and positively correlated with the pontomedullary A5(NA) nucleus, it should be easy to assume that the drug should act at this latter location rather than at the hypoactive A6(NA) nucleus. Desipramine should inhibit the uptake of NA at the A5(NA) but not at the A6(NA) axons, which were not releasing noradrenaline in these patients.[112] Thus, the drug would accentuate the A5(NA) over A6(NA) predominance that underlies diseases depending on the neural sympathetic hyperactivity.[113-115] In our long experience dealing with this issue, this obstacle can be avoided with the addition of clonidine to the NA-uptake inhibitor (desipramine).[116-118] This alpha-2 agonist is able to inhibit the hyperactive A5(NA) neurons but not the hypoactive A6(NA) and C1(Ad) neurons.[112,119,120] This effect will result in the restoration of the balance between A5(NA) vs A6(NA) and A5(NA) vs C1(Ad) nuclei.[119-124] The above neuropharmacological strategy has allowed us the successful treatment of thousands of patients affected by neural sympathetic predominance.

According to the above, the restoration of the A5(NA) vs A6(NA) balance results in the disinhibition of the adrenal sympathetic activity, which is minimized in patients affected by neural sympathetic predominance.[98,125,126] This target is reached through the above mentioned therapeutical strategy because the A5(NA) and the Cl(Ad) medullary nuclei interchange inhibitory axons.[127,128] The C1(Ad) axons release adrenaline at the A5(NA) nucleus whereas axons from the latter release NA at the former

nuclei. Both nuclei are crowded by alpha-2 inhibitory autoreceptors, thus the predominance of one of them should inhibit the activity of the other nucleus. These mechanisms explain the low levels of adrenaline registered in the plasma of patients affected by neural sympathetic overactivity.[59,129,130]

The recovery of the activity of the A6(NA) neurons, which are also inhibited by the overwhelming preponderance of the A5(NA) neurons, merits additional comments. The A6(NA) nucleus is integrated by some 36,000 NA neurons (more than any other NA nuclei located at the CNS level).[131-133] Axons of the A6(NA) nucleus inhibit the A5(NA) neurons and modulate the C1(Ad) medullary nuclei. The neurophysiological recovery of the A6(NA) + C1(Ad) neurons registered after the above mentioned therapeutical approach helps to explain the disappearance of symptoms and the normalization of the neuroautonomic disorders registered in patients affected by diseases underlied by neural sympathetic overactivity. In addition to the above, the well-known physiological minimization of the adrenal sympathetic activity registered in elderly people is highly reverted by the same neuropharmacological strategy.[130-134] Furthermore, the neural sympathetic predominance registered in elderly people helps to understand the cause of all types of pathophysiological phenomena frequently registered in them (psychological, cardiovascular, respiratory, sleep disorders, etc). Special mention should be devoted to the psychological disturbances. With respect to this, it should be known that all types of psychotic syndromes are underlied by a low number of A6(NA) neurons. This deficit is an inborn phenomenon in psychotic patients and a secondary disorder (because of involution) in patients affected by senile dementia. With respect to the above, we were the first to demonstrate that neural sympathetic overactivity underlies schizophrenia.[17-19,135] At the present time, it has been definitely demonstrated that patients affected by this psychiatric disorder have a greatly reduced number of A6(NA) neurons. This factor would explain the A5(NA) + neural sympathetic predominance that they also present.[95,136-138]

Not only catecholamines but also indolamines were assessed in the present study. The results obtained ratified others showing that patients underlied by neural sympathetic overactivity present with raised p-5HT plus lowered tryptophan circulating values. With respect to this, it has been demonstrated that the former is associated to the predominance of the median raphe (MR) over the dorsal raphe (DR) serotonergic nucleus, whereas the second disorder parallels the exhaustion of the DR(5HT) nucleus.[12,23]

Group A (Patients Showing Adrenal Sympathetic Predominance)

These patients presented lowered NA plus lowered NA/Ad plasma ratio, plus enhancement of both the SBP/DBP ratio and HR. These parameters were normalized after the desipramine administration. The facts showing that NA/Ad values were found to be positively correlated with the DBP values at the last 3 post-drugs periods support the postulation that the drug enhanced neural sympathetic activity in these patients. Although DA plasma values were also increased by the administration of the drug, no DBP changes were registered in these patients. This phenomenon should depend on the fact that in these patients, plasma DA arises from the adrenal glands source rather than from the under-active sympathetic nerves. The above postulation is supported by the significant positive (and not negative) correlations registered between Ad and

DA at the 4 post-drug periods.

Our findings showing that IM desipramine was also able to revert not only the decreased NA/Ad ratio but also the increased f5HT/p5HT ratio, reported before the neuropharmacological therapy, in patients affected by adrenal sympathetic predominance (Group A patients) might be explained by the enhancement of the neural sympathetic activity. Furthermore, the normalization of the raised f-5HT plasma levels registered in these patients was paralleled by the reduction of the increased platelet aggregability reported in them, which should be triggered by the raised Ad plasma levels that we found in these patients before the administration of the drug.[86,102] These peripheral effects provoked by the acute IM administration of the drug strongly suggest that the C1(Ad) over A5(NA) predominance was reverted.

Summarizing, the IM injection of 10 mg of desipramine provoked the enhancement of neural sympathetic activity in normal subjects and patients affected by both adrenal sympathetic and neural sympathetic predominance. Maximal effects were registered in the latter group whereas minimal but significant effects were observed in the other type of patients. Neuropharmacological therapy addressed to normalize the 2 types of CNS + ANS disorders was able to trigger the disappearance of both clinical symptoms as well as the physiological disturbances which underlie them. Finally, we discussed the relevance of our results with this type of neuropharmacological therapeutical strategy in the clinical practice.

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