In Vivo Evidence That Endogenous Dopamine Modulates Sympathetic Activity in Man

Massimo Mannelli, Lucia Ianni, Chiara Lazzeri, Walter Castellani, Cinzia Pupilli, Giorgio La Villa, Giuseppe Barletta, Mario Serio, Franco Franchi

Abstract—Dopamine receptors type 2 (D2)-like receptor blockers cause an increase in the norepinephrine response to intense physical exercise. However, during intense physical exercise, D2-like antagonists also cause an increase in the epinephrine response, which itself might cause an increase in plasma norepinephrine through the activation of β2 presynaptic receptors. Therefore, we evaluated the effect of domperidone, a D2-like antagonist, on the norepinephrine response to physical exercise in 6 Addison patients (3 were adrenalectomized and 3 had adrenal tuberculosis). In these patients, the norepinephrine increase observed during exercise was significantly higher after the administration of domperidone than a placebo (F=4.328; P<0.001). Because peripheral plasma norepinephrine does not reflect the sympathetic tone to the heart accurately, we evaluated the effect of domperidone administration (20 mg orally) on the sympathovagal balance, which was measured by the ratio between the high- and low-frequency components of heart rate variability, in 9 normal volunteers in the supine and sitting positions. When compared with placebo, domperidone caused a significant increase in the low/high frequency ratio (P<0.05) in the sitting position without modifying basal and stimulated norepinephrine plasma levels or blood pressure. These data support a role for endogenous dopamine in modulating norepinephrine release by human sympathetic nerves in vivo. (Hypertension. 1999;34:398-402.)

Key Words: dopamine ■ plasma ■ norepinephrine ■ heart

Neuronal dopamine receptors are widely distributed in the central and the peripheral nervous system at different levels. In vitro and in vivo studies have clearly demonstrated that the activation of these receptors leads to a decrease in catecholamine release. Whether endogenous dopamine plays a modulatory role on sympathetic nerve terminals through these receptors was investigated with Dopamine receptors type 2 (D2)-like receptor antagonists.

In previous studies, we demonstrated that D2-like antagonists do not modify plasma norepinephrine (NE) in humans when administered in conditions of rest or mild sympathetic stimulation, which is achieved by standing, a cold pressor test, or hand-grip. During intense sympathetic activation, as induced by physical exercise performed up to 80% of maximal oxygen consumption (VO2 max), D2-like antagonists cause a larger increase in plasma NE than placebo. These data suggest that during a high degree of sympathetic stimulation, endogenous dopamine, which is coreleased by nerve endings, increases in the synaptic cleft to a level sufficient to modulate and inhibit NE release.

However, during a high degree of sympathetic stimulation, the inhibition of D2-like receptors located in the chromaffin cells of the adrenal medulla also causes an increase in plasma epinephrine. Therefore, the greater increase of plasma NE observed during intense physical exercise in subjects receiving domperidone (DMP), a D2-like receptor antagonist, could be due, at least in part, to the facilitatory effect exerted by epinephrine on presynaptic β2 receptors and/or to the release of NE from the adrenal medulla. To rule out this possibility, we evaluated the effect of DMP on the NE response to physical exercise in 6 patients affected by primary adrenal failure also involving the medullary function.

Plasma NE, as measured in samples drawn from a peripheral vein, is an indicator of overall sympathetic activity and does not represent a reliable index of sympathetic activation in distinct districts, such as the heart, kidney, or gut. Therefore, the lack of any modification in plasma NE does not exclude the possibility that endogenous dopamine plays a modulatory role on cardiac sympathetic nerve terminals. Because power spectral analysis of heart rate variability (HRV) is a noninvasive and reliable technique for evaluating autonomic modulation to the heart, we used it to study the effect of D2-like receptor antagonists on the cardiac sympathovagal balance in normal volunteers.

Methods

The protocols of the study were approved by the Ethical Committee of the Department of Clinical Pathophysiology, and each participant gave her or his informed consent to the study.
Catecholamine Response to Exercise in Patients With Primary Adrenal Failure

The clinical data for each patient are reported in Table 1. Each patient was on glucocorticoid and mineralocorticoid replacement therapy. Two patients (patients 1 and 2) were also on thyroid replacement therapy. Before the study, patients had their \( \dot{V}O_2 \) max determined by performing an incremental bicycle ergometric test (Ergoline STS 800, Sensor Medics). The test started at the 30-W level and the load increased in steps of 10 W every 3 minutes until exhaustion. During the test, ECG was continuously monitored (Cardioline) and blood pressure (BP) was measured by sphygmomanometry. Gas exchange (\( O_2 \) and \( CO_2 \)) was monitored breath by breath with a gas analyzer (MMC 4400TC gas analyzer, Sensor Medics). The \( \dot{V}O_2 \)max was then used to calculate the submaximal \( V_0_2 \) levels.

Each patient was studied according to a double-blind, randomized protocol with a crossover design on 2 different occasions (placebo versus DMP) at least 7 days apart. Subjects were studied as outpatients, starting in the morning between 9 AM and 10 AM; they were fasting and had abstained from tobacco, tea, coffee, and cola for at least 12 hours.

The test was performed in a room with a constant temperature of 21°C. Each patient had an antecubital vein cannulated. After 10 minutes of rest in the sitting position, each subject received a placebo or DMP (20 mg orally). Twenty minutes later, the ergometric exercise was started at 30% of \( \dot{V}O_2 \)max (constant cycling speed, 50 rpm) and increased by steps of 10% every 3 minutes up to 80% of the \( \dot{V}O_2 \)max. Afterward, the cycling activity was decreased to 30% of the \( \dot{V}O_2 \)max for 3 minutes and then stopped. Finally, each patient rested for an additional 14 minutes in the sitting position.

Blood samples for measuring catecholamines were drawn ~20 minutes before the administration of placebo or DMP; at the beginning of the exercise (time 0); during the last 30 seconds of the 60% (time 12), 70% (time 15), and 80% step periods (time 18); and after 3, 7, and 17 minutes of recovery. Plasma samples for measuring prolactin were drawn before placebo or DMP administration, at the beginning of the exercise, and at the end of the 80% step period.

HRV in Normal Volunteers

Nine normal volunteers (3 women and 6 men aged 29.5±2 years; range, 20 to 49 years) were studied in the morning, in a quiet room with a constant temperature (21°C); they were fasting and had abstained from coffee, tea, cola, and tobacco for ≥12 hours. Each subject was kept recumbent and had an antecubital vein cannulated and kept patent with 0.9% saline. BP was measured in the opposite arm every 3 minutes throughout the experiment with an automated apparatus (Dinamap). Electrodes for continuous ECG recording were placed on the chest, together with a strain-gauge device for measuring breathing movements. Both signals (ECG and respiratory waves) were acquired online on a personal computer and converted from analog to digital information by a specific program (Global Laboratory) at the speed of 300 samples/s for each channel. The principles of the software and the analysis were previously described. Briefly, stationary sections of data of the appropriate length were selected for analysis. The computer program first calculated the interval ta-chogram (ie, the series of consecutive R-R interval values) and saved them in memory. From sections of tachograms of 512 interval values, simple statistics (mean and variance) of the data were computed. The length of the tachogram was selected as the best compromise between the need for a large series and the need for obtaining stationary recordings. The program also calculated the autoregressive coefficients necessary to define the power spectral density estimate and to provide the number, amplitude, and center frequency of oscillatory components.

Low-frequency (LF) and high-frequency (HF) components, both expressed in normalized units (nU) (as recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology12), and their ratio (LF/HF) were calculated. Normalization was done by dividing each component by the total power of R-R interval variability, subtracting the power below 0.03 Hz, and multiplying this ratio by 100.

The ECG registration for HRV was performed after 20 minutes of equilibration in the resting position, for ≥10 minutes either in the resting or sitting position, and after the administration of either placebo or oral DMP (20 mg). At the end of each registration period, while the patients were still either in the supine or sitting position, blood samples were drawn for the measurement of catecholamines and prolactin.

Laboratory Assays

All samples were collected in ice-cold tubes that contained 100 μL of a solution of glutathione (195 mmol/L) and EGTA (236 mmol/L) and centrifuged at 4°C. The plasma was stored at −25°C until assayed. Plasma NE and epinephrine were measured in duplicate and in the same assay for each patient by a radioenzymatic method previously described14 and with CAT-A-Kit (Amersham) according to the method of Passon and Peuler.15 The sensitivity of the method was 0.12 nmol/L for NE and 82 pmol/L for epinephrine. The between-assay variability was 10% for NE and 7.5% for epinephrine. Plasma prolactin was measured in duplicate and in the same assay for each patient by an immunofluorimetric assay with DELFIA prolactin Kits (LKB).

Statistical Analysis

Statistical analysis was performed with repeated measures 2-way ANOVA according to the general linear model.16 The test was performed with either placebo or DMP as the within-subject factor and the steps of exercise as the between-subject factor. The same test was then performed with NE changes during exercise as the within-subject factor and placebo or DMP as the between-subject factor. P<0.05 was considered significant. Statistical tests were performed with SPSS, version 7.5 (SPSS Inc).

Results

All results are expressed as mean±SE.

Catecholamine Response to Exercise in Patients With Primary Adrenal Failure

NE plasma levels measured in Addison patients before, during, and after the ergonomic test and after the administra-
tion of placebo or DMP are shown in the Figure. Plasma NE increased significantly during exercise after the administration of either placebo or DMP. No differences were found in the resting plasma NE level between placebo and DMP experiments. However, during exercise, the increment in plasma NE was significantly greater after the administration of DMP than a placebo. In fact, the 2 curves were significantly different at repeated measure 2-way (exercise and drug) ANOVA (F=4.328; P<0.001; power of the test=0.977). Moreover, a multiple pairwise comparison by a t test with a Bonferroni correction showed that NE plasma levels at times 15 (P<0.05) and 18 (P<0.02) were significantly higher after the administration of DMP than a placebo. In addition, the increments in plasma NE were different between DMP and placebo (F=2.678; P<0.02). The increment between times 15 and 18 was significantly greater after DMP administration than after a placebo (P=0.03).

In each patient, plasma epinephrine was below the limit of assay sensitivity in basal conditions. Plasma epinephrine remained undetectable during exercise in patients 1, 4, and 6. It became measurable during exercise at 80% of VO2max in 3 patients as follows: patient 2, 109.3 pmol/L after placebo and 200.5 pmol/L after DMP; patient 3, 102.7 pmol/L after placebo and undetectable after DMP; patient 5, 213.1 pmol/L after placebo and 332.8 after DMP.

As expected, plasma prolactin significantly increased after DMP (F=17; P<0.001). In fact, the mean values measured at times −20, 0, and 18 minutes after placebo and DMP were, respectively, 257±67 and 290±54 μU/L (P=NS); 264±76 and 282±61 μU/L (P=NS); and 275±92 and 890±117 μU/L (P<0.0001).

**HRV in Normal Volunteers**

Data for HRV and BP measured in healthy volunteers after placebo and DMP are reported in Table 2. BP did not change after either a change in posture or drug administration. The R-R interval significantly decreased in the sitting position after both placebo (P<0.05) and DMP (P<0.05) administration, with no differences between the 2 treatments.

As expected, the LF component increased, the HF component decreased, and the LF/HF ratio increased in the sitting position after placebo (P<0.05) and DMP (P<0.05) administration. No differences were found in LF component and LF/HF ratio between placebo and DMP treatment in the supine position, whereas the LF component, the HF component, and their ratio measured in the sitting position were significantly different between placebo and DMP treatments (all P<0.05).

Basal plasma NE was similar after treatment with placebo and DMP (0.85±0.05 nmol/L versus 0.98±0.06 nmol/L; P=NS). In the sitting position, plasma NE rose significantly after placebo (P<0.05) and DMP (P<0.05) treatment, and no differences were found between the 2 (1.98±0.16 nmol/L versus 2.02±0.17 nmol/L; P=NS). Plasma prolactin increased significantly after DMP treatment (215±53 versus 409±112 μU/L, P<0.01).

**Discussion**

The results of this study support the presence of an in vivo dopaminergic modulation of NE release from human peripheral sympathetic nerve endings. In fact, the greater plasma NE response to exercise observed in Addison patients after DMP cannot be attributed to an indirect contribution of the adrenal medulla. In these patients, the undetectable or very low levels of stimulated plasma epinephrine demonstrate that the adrenal medulla is not responsible for this effect.

The finding that in Addison patients, plasma epinephrine became measurable, although at very low concentrations, agrees with the results of other studies and suggests the presence of extra-adrenal sources of epinephrine in humans. In fact, phenylethanolamine N-methyltransferase (the enzyme that transforms NE into epinephrine) has been demonstrated in human tissues other than the adrenal medulla, such as the lung, kidney, and liver, although in much lower concentrations. Nevertheless, the concentrations of plasma epinephrine that we found in 3 of our patients at the peak of exercise are, in the mean, about 10-fold less than those we observed in normal subjects (1480±330 pmol/L after placebo and 2470±690 pmol/L after DMP); they are not sufficient to stimulate presynaptic β2-adrenergic receptors.

The source of endogenous dopamine acting on the presynaptic D2-receptors is still unknown. Free dopamine circulates in plasma, even during stressful conditions, at concentrations in the low nanomolar range and, therefore, it seems highly unlikely that it might activate presynaptic D2-receptors. These receptors can be activated only by locally produced dopamine and, therefore, a possible source might be the sympathetic neuron itself, which could corelease dopamine and NE. Indeed, in animal studies, experimental evidence exists for the presence of a nonprecursor dopamine pool in noradrenergic neurons.

The amount of dopamine released by noradrenergic neurons varies in different experimental conditions; it has been suggested that it increases with increasing sympathetic discharge. This hypothesis agrees with our data demonstrating that DMP, a D2-like receptor antagonist, enhances the release of NE only during intense physical exercise.

In our study, DMP administration, although it blocked D2-like receptors (as demonstrated by the increase in plasma prolactin), did not change plasma NE in Addison patients while they were in the sitting position or in normal volunteers in the supine or sitting positions. These data suggest that in

**Plasma norepinephrine concentrations (mean±SE) at rest (times −20 and 0), during graded exercise (times 12, 15, and 18), and during recovery (times 21, 25, and 35) after administration of placebo (continuous line) or DMP (dotted line) in 6 patients with primary adrenal failure. Curves are significantly different when analyzed with repeated measure 2-way ANOVA (F=4.328; P<0.001). *P<0.05 vs placebo; **P<0.02 vs placebo.
these conditions, the release of endogenous dopamine is insufficient to activate D2-like presynaptic receptors in the majority of sympathetic terminals. Nevertheless, DMP administration induced significant changes in the power spectral analysis of HRV in normal volunteers while sitting. HRV is an end-organ response determined by nerve firing, electrochemical coupling, adrenergic receptor sensitivity, postsynaptic signal transduction, and multiple neural reflexes.22

The finding that DMP causes a significantly different response in the LF/HF ratio with a change of posture suggests that the pharmacological block of D2-receptors modifies the sympathetic drive to the heart. The ultimate mechanism of this effect is speculative. To our knowledge, no data exist in the literature demonstrating that D2-antagonists can increase nerve firing or modify adrenergic-receptor sensitivity. However, in vitro23 and in vivo10 experimental evidence exists showing that they can enhance NE release from sympathetic nerve endings.

Therefore, although the final demonstration should rely on a DMP-induced increase in cardiac NE spillover, these data suggest that presynaptic D2-receptors also modulate NE release from cardiac nerve endings. The physiological importance of this modulatory mechanism in the human heart is difficult to establish. Nevertheless, it is worth mentioning that in the literature, some reports exist on sudden deaths in patients after the intravenous administration of large doses of DMP24,25 or metoclopramide.26 In all instances, the D2-blockers were administered as antiemetics to patients with malignancies who received cytotoxic drugs causing nausea. Our data can suggest that a pharmacological blockade of presynaptic D2-receptors might have contributed to cardiac arrhythmias by increasing the sympathetic tone in already-compromised hearts.

In conclusion, our study on patients with primary adrenal failure strongly supports the hypothesis that endogenous dopamine physiologically modulates NE release from human sympathetic nerve endings in vivo in response to severe exercise. In addition, the results of the study on HRV in normal subjects suggest that this modulation might be present in the sympathetic nerve endings of the heart during mild stimulation, such as a change in posture.

Acknowledgments
We thank Nadia Misciglia (Laboratory of Endocrine Unit) for her excellent technical assistance with the radioenzymatic assay of

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Drug + Tilt indicates the combined effect of the change of posture and of DMP administration. SBP indicates systolic blood pressure; and DBP, diastolic blood pressure. Values are mean±SE.

*P<0.05 vs supine.
plasma catecholamines and Dr Gianni Messeri (Central Laboratory, Azienda Ospedaliera Careggi) for prolactin measurements.

References

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_Hypertension_. 1999;34:398-402
doi: 10.1161/01.HYP.34.3.398

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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