Effects of Doxazosin and Propranolol on A_{2A} Adenosine Receptors in Essential Hypertension

Katia Varani, Roberto Manfredini, Valeria Iannotta, Cecilia Pancaldi, Elena Cattabriga, Canan Uluoglu, Pier Andrea Borea, Francesco Portaluppi

Abstract—A_{2A} adenosine receptors inhibit neutrophil adhesion and superoxide anion generation. The aim of the present study was to evaluate the effect of antihypertensive treatment with doxazosin or propranolol on the binding and functional parameters of A_{2A} adenosine receptors of lymphocytes and neutrophils in essential hypertensive patients. Two groups of previously untreated, essential hypertensive patients were studied. The mean affinity (K_d) and density (B_{max}) of adenosine receptors, by the A_{2A} selective radioligand [^{3}H]-ZM-241385 binding assays, and EC_{50}, by cAMP assays, were obtained first on no medication and a second time after treatment for up to 13 weeks with doxazosin (13 patients) or propranolol (8 patients). A third group of 15 healthy normotensive volunteers matched by age, sex, and body mass index was used as a control. Binding and functional parameters of the A_{2A} adenosine receptors were significantly higher in the 2 hypertensive groups than in controls (P always <0.0001), both in lymphocyte and neutrophil membranes. After treatment with propranolol, the binding parameters did not change significantly, whereas after treatment with doxazosin, K_d, B_{max}, and EC_{50} values returned to control levels. In never-treated essential hypertensive patients, lower affinity, higher density, and impaired function of A_{2A} adenosine receptors are present. The binding and functional parameters of A_{2A} adenosine receptors appear to be normalized after treatment with doxazosin but not with propranolol. **(*Hypertension*. 2002;40:909-913.)**

Key Words: adrenergic receptor blockers ■ antihypertensive agents ■ hypertension, essential ■ receptors, adenosine ■ assays

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Studies of the site of action of doxazosin indicate a selectivity for postjunctional α_1-adrenergic receptor, which is the primary mediator of the pressor effects of noradrenalin.[1] Doxazosin, however, reduces the overall cardiovascular risk of essential hypertensive patients[2,3] beyond its blockade of α_1-adrenoceptor–mediated vasoconstriction. We recently documented that treatment with doxazosin restores normal findings in the binding assays of platelet α_2-adrenoceptors of essential hypertensive patients.[4]

Propranolol exerts its antihypertensive action not only through β-adrenergic receptor blockade but also through a variable combination of effects on renin secretion, catecholamine release from adrenergic neurons, baroreceptor sensitivity, central nervous system control of arterial pressure, autoregulation of the circulation, and NO and prostaglandin synthesis.[5] Also, the benefits of improved survival and reduced reinfarction rate in coronary artery disease is probably multifactorial, with the antiischemic, antihypertensive, and antiarrhythmic effects of the drug all playing a role.[6,7]

Adenosine exerts its biological effects at the cardiovascular level through the activation of specific membrane receptors as A_1, A_{2A}, A_{2B}, and A_3 found in leukocytes, neutrophils, endothelial cells, smooth muscle cells, and cardiomyocytes.[8] Adenosine has potent cardioprotective effects via many different pathways, including reduction of the release and activity of catecholamines and increased blood flow.[9] Through interaction with A_{2A} receptors, adenosine is involved in platelet antiaggregator effects, neutrophil antiinflammatory responses,[10] and inhibition of cytokines release known to play a pathogenic role in chronic heart failure.[11] It has also been reported that adenosine, by means of interaction with adenosine receptors, inhibits α_1-adrenoceptor responses to many physiological and pathologic stimuli.[12–16] In view of the accumulating evidence of interactions between adenosine and the sympathetic system, the present study was designed to examine whether any changes in binding and functional characteristics of adenosine receptors are present in essential hypertension, and to evaluate the effect on adenosine receptors of antihypertensive treatments with doxazosin or propranolol.

Methods

The Ethics Committee of the University of Ferrara approved the study. After written informed consent was obtained from each

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subject and patient, the study was conducted in 2 groups of never-treated essential hypertensive outpatients: group 1 with 13 (8 male) patients and group 2 with 8 (5 male) patients. A control group of 15 (9 male) healthy subjects was also studied. The 3 groups were matched by sex, age, and body mass index (calculated by dividing body weight by the square of height). They had a mean (95% confidence limits) age of 41.4 years (37.3 to 45.5 years), 41.1 years (36.5 to 45.8 years), and 41.6 years (38.0 to 45.2 years) and a body mass index of 22.5 kg/m^2 (20.9 to 24.2 kg/m^2), 22.2 kg/m^2 (20.6 to 23.9 kg/m^2), and 22.1 kg/m^2 (20.8 to 23.4 kg/m^2), respectively, with no significant difference between groups (P always >0.65). All patients had a sitting systolic blood pressure >140 and <180 mm Hg or diastolic blood pressure >90 and <110 mm Hg as the mean value of 3 sphygmomanometer determinations taken between 9:00 and 11:00 AM on at least 3 separate occasions in the previous 4 weeks, in accordance with guidelines.[17] All patients underwent routine tests to rule out secondary hypertension, hypertensive target organ damage, or concomitant diseases. All patients and subjects were white nonsmokers.

Each patient was studied for a maximum of 13 weeks, consisting of a titration period of up to 4 weeks and 9 weeks of maintenance treatment. Group 1 patients were started on 1 mg doxazosin daily, taken at bedtime, whereas group 2 patients were started on 40 mg propranolol daily. The dose of doxazosin was doubled at weekly intervals, whereas propranolol was increased in weekly steps of 40 mg, until either the average seated diastolic pressure was <90 mm Hg and had fallen by at least 10 mm Hg from the baseline level, or a maximum daily dose of 8 mg doxazosin or 120 mg propranolol had been reached. At the end of the titration period, the mean daily dose was 6.2 mg (4.6 to 7.7 mg) doxazosin or 95 mg (64 to 126 mg) propranolol.

After the titration phase, there was a maintenance period of 9 weeks on the same dose of doxazosin or propranolol, at the end of which patients were seen again. Blood pressure was recorded at each visit.

Control subjects and hypertensive patients underwent a first laboratory study at baseline. Hypertensive patients and control subjects had a second laboratory study at the end of treatment and 14 weeks after the first study, respectively. Peripheral venous blood samples (50 mL) were obtained in each occasion between 9:00 and 11:00 AM.

[^{3}H]-ZM-241385 binding assays and measurement of cAMP levels in human lymphocytes and neutrophils were performed with the methods previously described.[18,19]

Data from saturation experiments and from cAMP assays were analyzed using the computer programs LIGAND[20] and Prism (Graph PAD), respectively. Statistical analysis of differences between groups was performed with nonparametric Mann-Whitney test for paired or unpaired data as appropriate. Differences were considered significant at a value of $P<0.05$. All data are reported as mean and 95% confidence limits.

Results

During treatment with doxazosin or propranolol, no patient experienced side effects that precluded completion of the study. At the end of the titration period, 3 patients under treatment with doxazosin (2 patients) and propranolol (1 patient) were still hypertensive (average seated diastolic pressure >90 mm Hg). Their diastolic pressures were between 90 and 100 mm Hg at maximum dose. Hence, they were continued in the study.

Mean systolic and diastolic blood pressure values are reported in Table 1 for the normotensive control group, studied twice 14 weeks apart, and for the 2 hypertensive groups studied both before and after treatment. In the control group, blood pressure was found unchanged from the first to the second study ($P=0.23$ and $P=0.28$ for comparison of systolic and diastolic values, respectively). Before treatment, blood pressure did not differ significantly in the 2 hypertensive groups ($P=0.80$ and $P=0.65$ for comparison of systolic and diastolic values, respectively). Treatment with the 2 drugs resulted in closely similar blood pressure levels ($P=0.80$ and $P=0.75$ for systolic and diastolic comparison respectively), with no significant difference from the normotensive values of the control group (P always >0.14).

TABLE 1. BP Values in the Normotensive Control Group Studied Twice and in the Hypertensive Groups Before and After Treatment With Doxazosin or Propranolol

Group	Systolic BP	Diastolic BP
Control group (n=15)*		
Study 1	128.1 (124.5–131.6)	81.9 (79.7–84.2)
Study 2	129.3 (125.0–133.7)	82.9 (80.3–85.6)
Group 1 (n=13)		
No treatment	156.9 (151.4–162.3)	98.4 (95.8–101.0)
After doxazosin	132.1 (127.7–136.5)	83.6 (79.8–87.5)
Group 2 (n=8)		
No treatment	155.0 (147.0–163.0)	97.4 (93.5–101.3)
After propranolol	132.4 (126.5–138.3)	84.1 (79.2–89.0)

Values are mean (95% confidence limits).
*Control group was studied twice, 14 weeks apart.

Table 2 shows the binding and functional parameters of A_{2A} adenosine receptors obtained twice in the normotensive control group, and before and after treatment with doxazosin or propranolol in the 2 hypertensive groups. The saturation curves of control subjects, untreated hypertensive patients, and hypertensive patients treated with doxazosin or propranolol are represented in Figure 1 for lymphocyte membranes and in Figure 2 for neutrophil membranes. The results of the experiments of stimulation by 5′-*N*-ethylcarboxamidoadenosine (NECA) of cAMP levels are reported in Figure 3 for lymphocyte and in Figure 4 for neutrophil membranes.

Closely similar results were observed in experiments performed on lymphocyte and neutrophil membranes. No significant differences in K_d, B_{max}, and EC_{50} values were found between the first and the second study (P always >0.30) performed in the control group. Before treatment, K_d, B_{max}, and EC_{50} values were significantly higher in the 2 hypertensive groups compared with the corresponding values in the control group (P always <0.001). After treatment with doxazosin, K_d, B_{max}, and EC_{50} values did not differ significantly from control values (P always >0.11), whereas after treatment with propranolol, the binding and functional parameters of A_{2A} adenosine receptors did not change significantly (P always <0.001 versus control).

Discussion

The present study describes, for the first time, the changes in the density and affinity of A_{2A} adenosine receptors in human lymphocyte and neutrophil membranes of uncomplicated essential hypertensive patients studied before any

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TABLE 2. Binding and Functional Parameters of the A_{2A} Adenosine Receptors in the Normotensive Control Group and in the Hypertensive Groups Before and After Treatment With Doxazosin or Propranolol

	Lymphocyte Membranes		Neutrophil Membranes		EC_{50} (nM)	
Subjects	K_D (nM)	B_{MAX} (fmol/mg protein)	K_D (nM)	B_{MAX} (fmol/mg protein)	Lymphocytes	Neutrophils
Control group						
Study 1	1.32 (1.23–1.41)	63 (56–69)	1.36 (1.07–1.65)	72 (66–78)	202 (179–224)	165 (142–188)
Study 2	1.37 (1.25–1.50)	66 (57–74)	1.41 (1.16–1.66)	73 (65–81)	206 (178–233)	169 (141–197)
Group 1						
No treatment	2.62* (2.39–2.86)	152* (141–163)	3.16* (2.66–3.66)	169* (160–178)	300* (267–333)	287* (262–311)
After doxazosin	1.47 (1.24–1.71)	70 (65–74)	1.65 (1.34–1.96)	79 (72–86)	231 (179–282)	222 (165–279)
Group 2						
No treatment	2.70* (2.33–3.07)	146* (131–161)	2.76* (2.42–3.09)	166* (151–182)	286* (245–327)	280* (227–334)
After propranolol	2.79* (2.50–3.07)	147* (123–170)	2.97* (2.57–3.38)	164* (146–182)	296* (271–322)	305* (262–348)

Values are mean (95% confidence limits).
*P always <0.001 vs Study 1 and Study 2 of the control group.

antihypertensive drug administration and after treatment with doxazosin or propranolol.

The close similarity of the data we obtained in 2 different membrane preparations (lymphocyte or neutrophil membranes), and the linearity of the Scatchard plots reported in the insets of Figures 1 and 2, indicate that in our experimental conditions, a single class of binding sites was present in both lymphocytes and neutrophils. In addition, the close similarity of the data obtained in the 2 studies performed 14 weeks apart in the control group indicates the absence of a significant time effect on the binding parameters of adenosine receptors in normotensive subjects. Moreover, our data indicate that in essential hypertension, A_{2A} adenosine receptors have a lower affinity but a higher density than the level in normotensive subjects. The main finding, however, is that after treatment with the α-adrenergic blocker doxazosin, the binding and functional parameters of A_{2A} adenosine receptors show values similar to the ones obtained in normotensive controls. This is not the case after treatment with the β-adrenergic blocker propranolol. On the other hand, the changes in blood pressure levels induced by the 2 different drugs were found to be similar, indicating that the changes in adenosine receptors observed only after doxazosin cannot be ascribed to blood pressure lowering per se. Altogether, our findings seem to suggest that the restoration of normal binding parameters in these hitherto untreated hypertensive patients might be specific either for doxazosin or for α_1-receptor antagonists in general, although a word of caution is necessary before accepting such an interpretation, as our study was not placebo controlled.

To investigate whether the differences found in binding parameters of hypertensive versus normotensive subjects are followed by alterations in the effector system, we also evaluated the stimulation of cAMP levels by NECA, a typical A_{2A} adenosine agonist. As indicated by the significantly higher values of EC_{50}, the potency of NECA to increase cAMP formation was significantly decreased in untreated hypertensive subjects than in normotensive control subjects. Moreover, in patients treated with doxazosin (but not in those treated with propranolol), the EC_{50} values did not differ significantly from the levels observed in normotensive subjects, revealing that the functionality of the A_{2A} adenosine receptors tends to return to normal after

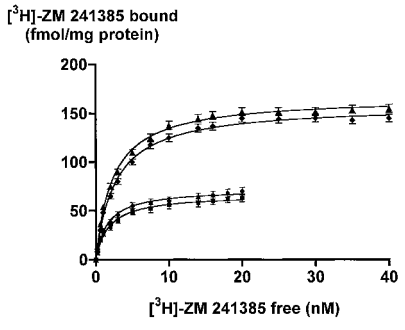


Figure 1. Saturation curves and Scatchard plots of [^{3}H]-ZM-241385 binding to lymphocyte membranes obtained from control subjects (■), untreated hypertensive patients (▲), and hypertensive patients treated with doxazosin (●) or propranolol (◇).

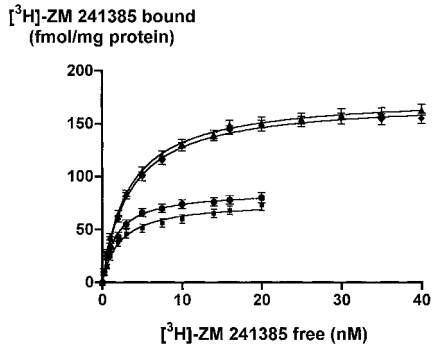


Figure 2. Saturation curves and Scatchard plots of [^{3}H]-ZM-241385 binding to neutrophil membranes obtained from control subjects (■), untreated hypertensive patients (▲), and hypertensive patients treated with doxazosin (●) or propranolol (◇).

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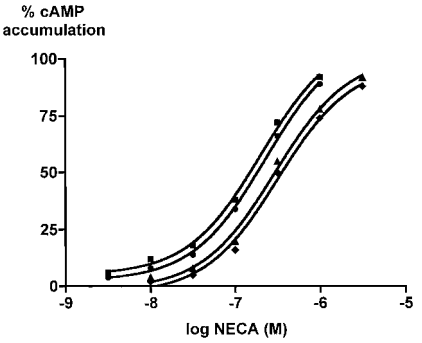


Figure 3. Stimulation by NECA of cAMP levels in lymphocytes obtained from control subjects (■), untreated hypertensive patients (▲), and hypertensive patients treated with doxazosin (●) or propranolol (◇).

pharmacological treatment with doxazosin, but not after treatment with propranolol.

Increased sympathetic nervous activity has been implicated in the pathophysiology of essential hypertension. The α_1-adrenoceptor–mediated vasoconstrictor component is enhanced in essential hypertension and relates to indices of increased adrenergic activity.[21] Increased sympathetic nervous activity appears to trigger cellular mechanisms that increase receptor density,[22] although normally an excess of stimulation produces receptor uncoupling and internalization leading to a decrease in receptor density.[23] On the other hand, this increase in density appears to be "compensated" by a lower receptor affinity. An inhibitory modulation of adenosine on α_1-adrenoceptor responses to many physiological and pathologic stimuli has long been suggested[12] and is supported by increasing experimental evidence,[13–16] suggesting that endogenous adenosine serves to minimize the potentially deleterious effects of α_1-adrenoceptor stimulation. Altogether, our findings suggest that changes in binding and functional characteristics of A_{2A} adenosine receptors are present and might contribute to altered cardiovascular regulation in essential hypertension, possibly through interactions with α_1-adrenoceptor–mediated responses to endogenous and exogenous stimuli. In fact, doxazosin normalizes the enhanced α_1-adrenoceptor–mediated vasoconstrictor component of hypertensive patients and stimulates adenosine receptors either directly or through induction of adenosine release from the muscle.[15] We observed in the present study that binding characteristics of adenosine receptors are also normalized after long-term treatment of essential hypertensive patients with this drug. Our data do not allow us further speculations on the possible mechanisms of such normalization. It should be noted, however, that because doxazosin has many pharmacological properties that are not fully explained by its blocking effect of α_1-adrenoceptors,[1] a direct action on adenosine receptors or other indirect effects cannot be excluded.

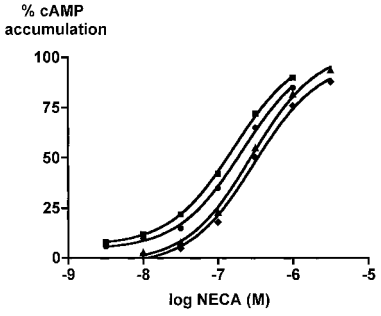


Figure 4. Stimulation by NECA of cAMP levels in neutrophils obtained from control subjects (■), untreated hypertensive patients (▲), and hypertensive patients treated with doxazosin (●) or propranolol (◇).

Perspectives

Any evidence derived from an adenosine receptor type studied in accessible tissues (including blood cells) needs to be weighed against the complex background of the normal structure and function of adenosine receptors subtypes in different human tissues. These are still incompletely characterized, and further studies are necessary before the pathophysiological significance of the findings of the present study can be understood.

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