Effect of the $\alpha_2$-Adrenergic Antagonist Yohimbine on Orthostatic Tolerance

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We studied the effect of yohimbine, a drug that inhibits presynaptic $\alpha_2$-adrenergic receptors and increases the neuronal release of norepinephrine from the central and sympathetic nervous systems, on tolerance to cardiovascular stress in 10 untrained, healthy subjects. Using radioligand binding of tritiated yohimbine to platelets, these subjects were found to have a normal complement of $\alpha_2$-adrenergic receptors (174±18 [±SEM] receptors/platelet) with normal $K_d$ (1.93±0.17 nmol/l). Lower body negative pressure was used to test responses to cardiovascular stress in the subjects after they received either placebo or 20 mg yohimbine. Graded lower body negative pressure from 0 to $-40$ mm Hg significantly decreased systolic blood pressure from 116±3.7 to 106±5.8 mm Hg, increased heart rate from 54±3 to 68±7 beats/min, decreased forearm blood flow from 1.8±0.21 to 1.36±0.25 ml/100 ml/min, and increased forearm vascular resistance from 55.76±12.1 to 77.26±15.8 mm Hg/ml/min. Yohimbine increased the blood pressure at rest and during lower body negative pressure, but these changes were not significantly different from values recorded from the individuals when they were given placebo. Compared with placebo, however, yohimbine significantly increased forearm blood flow at rest ($1.80±0.21$ vs. $2.66±0.31$ ml/100 ml/min, $p<0.05$) and during $-40$ mm Hg of lower body negative pressure ($1.36±0.25$ vs. $1.91±0.28$ ml/100 ml/min, $p<0.05$). We also found that yohimbine significantly increased the plasma insulin concentration in these fasted subjects ($9.4±2.4$ vs. $14.5±1.4$ ng/ml, $p<0.05$) without inducing hypoglycemia. Because this agent increases forearm blood flow, yohimbine might be useful in treating the orthostatic hypotension and ischemic vascular disease that results from the autonomic insufficiency common in patients with diabetes mellitus. (*Hypertension* 1990;15:877–880)

The $\alpha$-adrenergic receptors are found on presynaptic neurons of the central nervous system, the peripheral nervous system, and blood vessels. The $\alpha_2$-adrenergic receptors are found mainly on blood vessels, and when stimulated by endogenous ligands such as norepinephrine, cause vascular smooth muscle to contract and vascular resistance and blood pressure to increase. The $\alpha_2$-adrenergic receptors are found on platelets, in the vasculature, and on presynaptic neurons. Activation of presynaptic $\alpha_2$-adrenergic receptors results in a decrease in catecholamine release from nerve terminals and the adrenal medulla and a decrease in blood pressure. Alternatively, antagonism of presynaptic $\alpha_2$-adrenergic receptors results in an increase in catecholamine release.1–3

Because increasing circulating and central catecholamines increases blood pressure, we hypothesized that pharmacological agents that increase catecholamine release could increase tolerance to cardiovascular stress experienced by patients with orthostatic hypotension or by fighter pilots who experience increased gravitational force (+Gz). We measured the effect of the oral $\alpha_2$-adrenergic receptor antagonist yohimbine on the blood pressure response to the unloading of cardiovascular baroreceptors with graded lower body negative pressure (LBNP).

**Methods**

Nine healthy men and one woman, all 22–46 years old, were recruited for these studies. These protocols were approved by our Institutional Committee on the Use of Humans in Research, and all subjects gave written informed consent. All volunteers passed a US Air Force class III physical examination and were taking no medications. The subjects fasted for at least 4 hours before the experiment, and each individual subject was studied at the same time of day.
We determined platelet $\alpha_2$-adrenergic receptor number and binding constants for each subject in duplicate as previously described. Briefly, 30 ml blood was collected by venipuncture into EDTA vacutainer tubes. The plasma was centrifuged at 180g for 20 minutes, and an equal volume of isotonic buffer containing 50 mmol/l Tris-HCl, 100 mmol/l NaCl, and 5 mmol/l EDTA (pH 7.5) was added to the platelet-rich plasma. The platelets were then centrifuged at 17,000g, washed again, and then resuspended in the buffer. Platelet counts and size distribution were obtained from a model S Plus Coulter Counter (Coulter Corp.-Sci. Instrs., Hialeah, Florida). One hundred fifty microliters of platelets (approximately $5 \times 10^7$ platelets) were incubated with 50 $\mu$l of increasing concentrations of $[^3H]$ yohimbine (1.0-20.0 nM, New England Nuclear, Boston, Massachusetts) with a specific activity of 84.5 Ci/mmol. The reaction was brought to a final volume of 250 $\mu$l with buffer or phentolamine mesylate (2 mM, Regitine, Ciba, Summit, New Jersey) to block nonspecific binding. The reaction was allowed to proceed at room temperature for 45 minutes, and then the reaction was stopped by the addition of ice-cold buffer and rapid filtration on Whatman chromatographic fiberglass filter paper (Whatman LabSales Inc., Hillsboro, Oregon). The filter papers containing the platelet-bound yohimbine were placed in 5.0 ml Insta-Gel Scintillation Fluid (United Technologies, Columbia, City, Indiana) and counted in a Hewlett-Packard Tricarb Scintillation Counter (Palo Alto, California). The amount of bound and free ligand was calculated, and the saturation binding isotherms were derived by using Scatchard analysis.

For LBNP, the subjects were encased to the waist in an LBNP chamber (Biomedical Engineering, University of Iowa) in a room with constant temperature ($72^\circ \pm 3^\circ$ F). The subjects blindly received 20 mg yohimbine orally or placebo capsules 1.5 hours before beginning the study. A heparinized catheter was placed in the right antecubital vein for blood drawing. Blood pressure and heart rate were frequently monitored electronically using an automated cuff method and recording device (Dinamap Vital Signs Monitor model 1160, Critikon, Cleveland, Ohio). Forearm blood flow in the left arm was measured with a mercury-in-Silastic strain gauge plethysmograph (Hokansan Instruments, Issaquah, Washington) and Gould amplifier and recorder (Gould Electronics, Cleveland, Ohio).

After resting for 30 minutes in the LBNP chamber (and 1.5 hours after randomly receiving placebo or yohimbine), baseline values were obtained. LBNP at −10 mm Hg was initiated and abruptly increased by 10 mm Hg every 5 minutes until the LBNP reached −60 mm Hg, or until the subject had symptoms of impending syncope or a decline in blood pressure to a value less than 80/50 mm Hg. All subjects tolerated the experimental protocol well, and there were no untoward reactions.

We also determined plasma norepinephrine, insulin, and glucose levels in individuals after placebo and yohimbine administration. Plasma norepinephrine was measured with high-performance liquid chromatography using electrochemical detection. Insulin levels were measured by radioimmunoassay, and glucose concentration was measured with an automated analyzer.

Data were analyzed using the MIDAS statistical program from the University of Iowa (Ann Arbor, Michigan). The data were analyzed with repeated measures of analysis of variance with two repeated factors, time and drug effects. With this analysis, an interaction is possible between time and drug. When an interaction is statistically significant, the response to different drugs is different throughout time. Because no interactions were present, we tested for overall drug differences and for the effect of increasing the magnitude of LBNP throughout time.

Results

Because physical training can influence the catecholamine response to various cardiovascular stresses such as orthostasis or $+G_z$ and because aerobic training can increase $\alpha_2$-adrenergic receptors, we first determined that we had a homogeneous subject population. As demonstrated in Figure 1, individuals in our study had normal $\alpha_2$-adrenergic receptors as demonstrated by the number of platelet receptors (174±18 [±SEM] binding sites/platelet) and normal dissociation constants ($K_d$) (1.76±0.17 nmol/l).

Although yohimbine tended to increase systolic blood pressure in the subjects at rest compared with values obtained when the subjects were given placebo, these changes did not reach statistical significance at levels of $p$ values less than 0.05. At rest,
yohimbine did not significantly affect the diastolic blood pressure (64±3 vs. 65±3 mm Hg, p=NS) from control values. Heart rate, however, significantly increased (54±3 vs. 60±3 beats/min, p<0.05). Graded LBNP from 0 to -40 mm Hg significantly decreased systolic blood pressure, increased heart rate, and decreased forearm blood flow. Forearm blood flow was consistently higher at baseline and during LBNP in subjects receiving yohimbine compared with subjects given placebo (Figures 2 and 3).

Twenty milligrams of yohimbine did not consistently increase plasma norepinephrine in all subjects (157±26 vs. 270±99 pg/ml, control vs. yohimbine, p=NS). As demonstrated in Figure 4, however, yohimbine did increase the mean plasma insulin concentration from 9.2±1.2 to 12.5±1.0 ng/ml (p<0.05) without affecting plasma glucose concentration (86±14 vs. 81±12 mg/dl, p=NS).

**Discussion**

The characterization of platelet α2-adrenergic receptors has been used as a less invasive measurement of neuronal α2-adrenergic receptors. It has been demonstrated that endurance training increases the number of α2-adrenergic receptors.1 Because endurance training can decrease the ability of subjects to withstand the unloading of cardiovascular baroreceptors,4-5 we confined our study to a homogeneous, untrained population. The receptor number and binding constant found in our subjects is similar to those reported in other healthy, untrained subjects.1 Twenty milligrams of yohimbine was well tolerated by our subjects. Yohimbine did not consistently increase plasma norepinephrine concentrations. Because norepinephrine is rapidly taken up by neurons and metabolized by monoamine oxidase and catechol-O-methyl transferase, plasma norepinephrine does not necessarily correlate with the turnover of central or peripheral catecholamines. It has been demonstrated, however, that 20 mg yohimbine can significantly increase the turnover of plasma norepi-
nephrine as reflected in elevated plasma levels of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol. It is possible that the local concentration of norepinephrine at the neuroeffector junction is sufficiently increased to be physiologically significant without increasing the circulating concentration of plasma norepinephrine.

LBNP redistributes blood from the central blood volume to the lower extremities (a hemodynamic effect similar to +Gz), and this decrease in venous return is sensed by baroreceptors. The low pressure cardiovascular baroreceptor reflex system that is activated by low pressure baroreceptors activates higher levels of LBNP (greater than −20 mm Hg) can result in a reflex increase in vascular contractions and a subsequent increase in blood pressure mediated by activation of the sympathetic nervous system and inhibitory vagal afferents from the medullary vasomotor center.

During activation of low pressure and high pressure baroreceptors by LBNP, subjects maintained higher blood pressure when given yohimbine; however, the increases in blood pressure did not reach statistical significance (p=0.08). It has been demonstrated that yohimbine can increase systemic venous capacity in dogs, and it is possible that an increase in venous capacity by yohimbine in humans could attenuate the direct effects of yohimbine on the neurovascular junction. When given yohimbine, however, subjects had higher heart rates and greater forearm blood flow during graded LBNP. The increase in forearm blood flow might explain the less-than-significant effect of yohimbine at maintaining blood pressure during LBNP. It has been demonstrated that vasoconstriction is mediated by α₂-adrenergic receptors in human digits, and yohimbine increases digit blood flow. The increase in forearm blood flow induced by yohimbine is most likely due to a unique complement of α₂-adrenergic receptors on the arterial smooth muscle of the extremities.

Because patients with diabetes frequently have ischemic extremities, because inhibition of α₂-adrenergic receptors inhibits glucose-stimulated insulin release, because yohimbine was recently reported to raise plasma insulin concentrations in the dog, and because yohimbine can help counteract the orthostatic hypotension commonly found in patients with diabetes mellitus, we also measured plasma insulin concentrations in our fasted subjects after placebo and yohimbine administration. Yohimbine did increase plasma insulin concentrations without significantly affecting plasma glucose concentrations.

Yohimbine was inconsistent in decreasing the hypotensive response to LBNP in our untrained subjects. It has been previously reported that yohimbine can increase tolerance to orthostatic stress in patients with primary autonomic insufficiency, and the response to yohimbine in our subjects during LBNP was heterogeneous. Individual differences in response to yohimbine might be secondary to differences in receptor binding induced by genetic polymorphism of the α₂-adrenergic receptor. It remains to be determined whether yohimbine will be useful in decreasing the hypotensive response to LBNP in trained individuals that have a greater complement of α₂-adrenergic receptors. Yohimbine might be useful in preventing orthostatic hypotension in some diabetics with autonomic insufficiency or in some aviators exposed to rapid onset +Gz.

References


Key Words • yohimbine • lower body negative pressure • human studies • catecholamines • orthostatic hypotension • insulin • adrenergic receptors
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