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Yohimbine – facilitated acoustic startle reflex in humans

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Abstract. Preclinical studies have suggested the acoustic startle reflex (ASR) may be a useful animal model to investigate the neurochemical basis of anxiety and fear states. This work has revealed that the anxiogenic alpha-2 receptor antagonist, yohimbine, increases the amplitude of the ASR in laboratory animals. The present investigation evaluated the effects of yohimbine on the ASR in healthy subjects. Seven healthy subjects received IV yohimbine (0.4 mg/kg) or saline placebo on two separate days in a randomized double blind placebo control design. A trial of 2 tone frequencies with varied intensity (90, 96, 102, 108, 114 dB) white noise, instantaneous rise time, was delivered binaurally through headphones. Tones were delivered every 25–60 sec, for a 30 ms duration. Startle testing was done 80 minutes post infusion and lasted 15–20 minutes. Sign rank testing indicated yohimbine caused an overall increase in startle amplitude, as well as significant augmentation of startle amplitude at 96, 102, 108, 114 decibels but not at the 90 dB intensity. Sign rank tests indicated a significant reduction of startle latency by yohimbine at only the 96 dB intensity. Significant correlations were observed between startle and peak anxiety, startle and plasma MHPG, peak anxiety and plasma MHPG. This study demonstrates in healthy human subjects an excitatory effect of yohimbine on the magnitude of the ASR and a decrease in its latency. In the context of the key role of this reflex in the alarm response, this finding adds to the array of documented behavioral, biochemical and cardiovascular effects of yohimbine in humans which support the relationship between increased noradrenergic function and anxiety states.

Key words: Yohimbine – Noradrenergic – Anxiety

The startle reflex is a ubiquitous, cross-species mammalian skeletomuscular response to a sudden exteroceptive stimulus (for review see Davis 1984). It is typically measured as a short-latency whole-body movement in rodents or an eyeblink in humans, because both are reliable and easily quantified (Davis 1984; Butler et al. 1990).

Preclinical studies in the rat indicate that the startle reflex is increased by both conditioned and unconditioned fear. For example, the amplitude of the startle reflex is increased when elicited in the presence of a stimulus previously paired with an aversive shock (Brown et al. 1951; Davis and Astrachan 1978; Davis 1989). This fear-potentiated startle effect is selectively decreased by anxiolytic compounds such as diazepam (Davis 1979) or buspirone (Kehne et al. 1988) and increased by anxiolytic compounds such as yohimbine (Davis et al. 1979) or the beta-carboline DMCM (Huzen and Slangen 1989).

Controlled clinical studies indicate that the eyeblink component of startle in humans also is potentiated by fear. Spence and Runquist (1958) and Ross (1961) reported that a visual stimulus paired with an aversive shock increased the amplitude of the eyeblink elicited by an airpuff to the temple. Vrana et al. (1988) found the acoustic startle reflex of healthy college students was potentiated when viewing "negative affect slides" (pictures of knives, spiders, mulitated bodies) compared to "positive affect slides" (beach scenes, babies, attractive models). Similarly, Scott et al. (1990) and Cook et al. (1991) reported potentiation of startle during aversive imagery (scenes of assault or attack), compared to pleasant imagery (scenes of pleasurable experiences). The data suggest that imagery related to emotions of anger, anxiety or fear potentiated the startle response while imagery associated with relaxed or pleasant emotions did not. Grillon et al. (1992) investigated the effects of fear and anticipatory anxiety on the startle response. Healthy subjects showed potentiated startle amplitude when anticipating electric shock compared to periods when no shock was anticipated.

A variety of drugs has been found to exacerbate anxiety in healthy subjects and anxiety disorder patients.

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Among the best studied agents is yohimbine, which elicits anxiety in healthy subjects and markedly exacerbates anxiety in patients with panic and post-traumatic stress disorders (Charney et al. 1984, 1987; Southwick et al. 1991). These anxiogenic effects of yohimbine appear related to activation of central noradrenergic systems because yohimbine is an alpha$_2$ adrenergic receptor antagonist which results in increased firing of the primary brain noradrenergic nucleus, the locus coeruleus, and brain norepinephrine turnover. Consistent with these data, in humans yohimbine increases plasma levels of the norepinephrine metabolite, MHPG, and cerebrospinal fluid levels of norepinephrine (Charney et al. 1984, 1987; Peskind et al. 1989).

Preclinical studies have shown that yohimbine elevates both baseline acoustic startle amplitude (Kehne and Davis 1985) and the magnitude of fear-potentiated startle in rats (Davis et al. 1979). The purpose of this preliminary investigation was to evaluate the effects of yohimbine on the eyeblink component of the acoustic startle reflex in healthy humans. Identification of an excitatory effect of yohimbine on human startle would provide further evidence of a relationship between noradrenergic hyperactivity and anxiety and alarm states in humans.

Materials and methods

Seven healthy male subjects were recruited from responses to advertisements and from referrals by other healthy subjects. They all gave voluntary written, informed consent for their participation in the study. The healthy subjects were determined to be free of mental disorder on the basis of a structured psychiatric interview, and none of the subjects reported a history of mental illness in first-degree relatives. The healthy subjects reported not taking any psychoactive medication for the 4 weeks prior to the study. The mean age of the subjects was 29 ($\pm$4) years. None of the healthy subjects reported a history of serious medical illness, and they all had normal results on physical examination, ECG, and laboratory tests of renal, hepatic, pancreatic, hematopoietic, and thyroid function.

Test days. Prior to each test day, subjects fasted overnight for 10 h and remained in the fasting state throughout testing. Each healthy subject participated in 2 test days (yohimbine and placebo). The subjects arrived on the Neurobiological Studies Unit at 8:30 A.M. on each test day. At that time, an intravenous line was placed to permit medication administration and blood sampling for plasma MHPG. A minimum of 2 h after insertion of the intravenous line, yohimbine (0.4 mg/kg) or saline was infused over 10 min. Startle testing began 80 min post-infusion. This study was part of a larger investigation. The 80-min delay was due to the requirements for repeated assessments of the behavioral and biochemical effects of yohimbine. As previously described, the degree of anxiety produced by yohimbine was assessed using a 100 mm visual analog scale (0 = no anxiety at all to 100 = extreme anxiety) 30 min prior to infusion and 30, 60, and 120 min following the infusion. Anxiety was defined as "a mental awareness of worry, apprehension, or dread, which is often, but not always, accompanied by physical feelings like jitteriness, tension, heart throbbing, breathlessness, etc." (Charney et al. 1984, 1987).

Blood was drawn for plasma MHPG determinations at 30 min and 15 min prior to infusion and at 40, 60, 90, 120, and 180 min post-infusion. Blood samples were kept on ice for a maximum of 1 h before separation of plasma in a refrigerated centrifuge. The plasma specimens then were frozen at −70° C until assay. Preparation of the sample for MHPG analysis was carried out according to a modified version of the method of Dekirmenjian and Maas (1974). Quantitation of the plasma-free MHPG level was carried out by selected ion monitoring, as described elsewhere, using a quadrupole mass spectrometer equipped with a gas chromatographic inlet system (Dekirmenjian and Maas 1974; Maas et al. 1976). Intra- and interassay coefficients of variation were 6% and 11%, respectively. To reduce the variance in method plasma speciments were assayed in duplicate. The subjects, research nurse, and laboratory staff were blind to the sequence of the yohimbine and placebo doses.

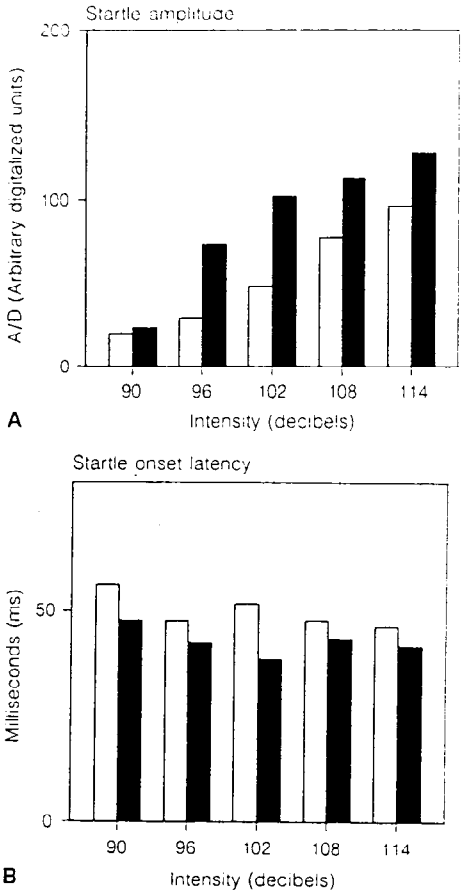

Fig. 1. A Effect of yohimbine on acoustic startle reflex (ASR) amplitude. Amplitude of EMG activity of the orbicularis oculi is recorded in arbitrary audio-digitalized units (A/D). *Bars* show the ASR amplitude at 90, 96, 102, 108 and 114 dB after yohombine administration *(black bars)* as compared to placebo *(white bars)*. Yohimbine significantly increased amplitude at 96, 102, 108, 114 dB compared to placebo ($P<0.02$; $P<0.2$; $P0.02$; $P<0.03$, respectively). (Subjects received intravenous infusion of 0.4 mg/kg yohimbine HCl vs placebo in a randomized, double blind fashion. Eighty minutes post-infusion the startle reflex was measured.) B Effect of yohimbine on acoustic startle reflex latency (the time to blink onset): Bars show in milliseconds latency after intravenous infusion of 0.4 mg/kg of yohimbine HCl *(black bars)* compared to placebo infusion *(white bars)*. Drug effect showed significance at the 96 dB intensity ($P<0.01$)

Acoustic startle reflex assesment. Eighty minutes post-infusion the startle was recorded with a commercially available startle system (SR-Lab; San Diego Instruments) in a sound-attenuated chamber. Subjects were seated in a comfortable chair which was kept in an upright position. Audioscopic assessment (Welsh Allyn) tested hearing at 500, 1000, 2000 and 4000 Hz. An audiologic exclusion criterion was any hearing loss greater than one frequency band in

one ear. No subjects were eliminated on the basis of the audiologic assessment. No hearing loss was noted in any of the subjects.

The orbicularis oculi electromyographic (EMG) activity was recorded with two disc electrodes (Ag-AgCl) placed 1 cm below and 1 cm from the external canthus of the right eye. The ground electrode was placed on the forehead. Impedence was kept below 8 Kohm. EMG activity was filtered (1–1500 Hz, digitized for 250 ms from onset of acoustic stimuli, rectified, and stored for off-line analysis.

The acoustic stimulus was a 30 ms burst of white noise with a near instantaneous rise time presented binaurally through headphones (Maico). Sets of acoustic stimuli were calibrated with a Sound Level Meter (Realistic) to five intensities; 90, 96, 102, 108, 114 dB(A). These stimuli were delivered to subjects over a background of 75 dB(A) white noise. Sound was calibrated by means of a 6 cc coupler in an artificial ear (Model EC-9A) and continuous noise.

Recording sessions commenced 180 s after background noise onset with an initial startle pulse (102 dB) which was not included in the data, followed by seven blocks of acoustic stimuli. Each block was composed of the five intensities presented in a pseudorandomized order every 45–60 s over a period of 15–20 min. Recording sessions ended 15 s after delivery of the 36th acoustic stimulus, at which time the background noise was turned off.

To analyze the blink reflex, the digital signal was smoothed by a rolling average of 5 successive points. Onset latency, the time to onset of the blink reflex, and peak amplitude (in arbitrary units) of the blink reflex were determined in the 21–95 ms following stimulus onset. Peak amplitude was calculated relative to a "baseline" value. This baseline value was calculated by taking the average of the minimum and maximum values recorded during the first 20 ms. The response criterion for a peak was set at 10 arbitrary units. Trials were rejected because of unstable EMG activity during the first 20 ms or failure to reach peak within 95 ms of onset latency. In the analysis of latency, trials were rejected on the basis of failure to blink. Only two trials were eliminated in two subjects in the analysis of the latency for failure to blink.

Statistical analysis. The data were entered into computer files and stored for nonparametric analysis due to the small size of this preliminary study and the lack of a normal distribution. To examine the effect of intensity on startle amplitude, the Friedman Fr test was used (equivalent to randomized block ANOVA) (McClave 1988). To examine the effect of yohimbine on startle amplitude and startle latency, sign rank tests were performed (test day placebo vs test day yohimbine difference in startle amplitude and latency) (SAS User's Guide 1985). In order to examine startle habituation, linear regression slopes of the startle amplitude data were calculated for each of the five stimulus Intensities across the seven test blocks. These slopes were tested for significant variance from zero under both placebo and yohimbine conditions. The effect of yohimbine on anxiety ratings and plasma free MHPG level was determined using sign rank tests. Sign rank tests were conducted to determine whether and at what time point yohimbine had different effects on these parameters compared to placebo (yohimbine-placebo differences of change from baseline at each time point). Pearson correlations were calculated to examine the relationship among the peak effects of yohimbine on startle, anxiety ratings, and plasma MHPG. Pearson correlations were calculated to examine the relationship among the peak effects of yohimbine on startle, anxiety ratings, and plasma MHPG.

Results

Figure 1A shows the mean amplitude of the startle reflex as a function of the intensity of the startle-eliciting stimulus after injection of saline (white bars) or yohimbine (black bars). Consistent with previous research, the population distributions for the amplitude of the eyeblink component of startle at the five stimulus intensities were not identical ($F=23.6$, chi squared 0.05@4 df=9.49). As shown in Fig. 1A, startle amplitude increased with sti-

Table 1. Effects of yohimbine and placebo on anxiety levels of healthy subjects

Group and drug	Anxiety level score (mm)									
			Change from baseline							
	Baseline		20 min		60 min		120 min		180 min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy subjects ($n=7$)										
Placebo	10.9	15.4	5.2	10.4	9.2	1.9	4.3	11.1	8.8	2.8
Yohimbine[a]	8.6	8.7	18.3	24.5	2.2	9.5	3.5	8.0	4.5	5.0

[a] Yohimbine, in comparison to placebo, increased anxiety at 20 min, as reflected by a change from Baseline, yohimbine vs placedo, sign rank test (s=12; $P<0.05$)

Table 2. Effects of yohimbine and placebo on plasma-free MHPG levels of healthy subjects

Group and drug	MHPG level (ng/ml)									
			Change from baseline							
	Baseline		40 min		60 min		120 min		180 min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy subjects ($n=7$)										
Placebo	4.1	1.2	0.4	1.4	0.2	1.3	0.6	1.8	1.3	2.0
Yohimbine[a]	3.7	1.1	1.4	1.5	1.4	1.8	1.5	1.2	1.2	1.3

[a] Yohimbine, in comparison to placebo, increased MHPG at 60 min, as reflected by a significant change from Baseline, sign rank test (s=12; $P<0.04$)

mulus intensity. Sign rank testing indicated yohimbine caused an overall increase in startle amplitude ($s=0.14$; $P<0.02$). Sign rank tests performed at the individual stimulus intensities demonstrated a significant augmentation of startle amplitude by yohimbine at 96 ($s=0.14$; $P<0.02$), 102 ($s=0.14$; $P<0.02$), 108 ($s=0.14$; $P<0.02$), 114 ($s=0.13$; $P<0.03$) decibels, but not at the 90 dB intensity ($P<0.84$).

Figure 1B shows the mean latency of the startle reflex as a function of the intensity of the startle-eliciting stimulus after injection of saline or yohimbine. Sign rank tests performed at the individual stimulus intensities demonstrated a significant reduction of startle latency by yohimbine at only the 96 dB intensity ($s=0.14$; $P<0.01$). No significant overall effect of stimulus intensity were noted.

The linear regression slopes of startle amplitude data were negative, indicating that habituation was present. The magnitude of the negative slope was similar during the yohimbine and saline placebo conditions, and the slopes did not differ significantly from each other. However, only the slope of the placebo day differed significantly from zero. These significant findings occurred at 90 dB ($P<0.01$), 96 dB ($P<0.01$), 102 dB ($P<0.01$) and 108 dB ($P<0.05$). These data suggested that the decremental response over time to a repeated stimulus was possibly delayed or diminished under the yohimbine condition as compared to the placebo condition for all but the 114 dB stimulus category.

Tables 1 and 2 show the anxiety level ratings and the MHPG values at the various time points before and after yohimbine or saline administration. Yohimbine, in comparison to saline placebo, significantly increased anxiety at 20 min ($s=11$; $P<0.05$) and MHPG at 60 min after the dose ($s=12$; $P<0.05$).

Significant correlations were observed between the following effects of yohimbine: startle elicited by 102 db and peak anxiety ($r=0.78$, $P<0.05$); startle elicited by 102 dB and plasma MHPG ($r=0.77$, $P<80.05$); and peak anxiety and peak plasma MHPG ($r=0.93$, $P<0.005$).

Discussion

Consistent with preclinical investigations, this study demonstrates in healthy human subjects, an excitatory effect of yohimbine on the magnitude of the acoustic startle reflex and a decrease in startle latency. The excitatory effect of yohimbine was statistically significant at all startle-eliciting test intensities except 90 dB. This may reflect a threshold effect and emphasizes the utility of test procedures that include a range of stimulus intensities. In the context of the key role of this reflex in the alarm response, this finding adds to the array of documented behavioral, biochemical, and cardiovascular effects of yohimbine in humans which support the relationship between increased noradrenergic function and anxiety states (Charney et al. 1984, 1987).

The facilitation of startle by yohimbine may be due to increased noradrenergic function in the spinal cord. In rats, yohimbine's excitatory effect on baseline startle appears to result from an increase in release of norepinephrine in the spinal cord (Kehne and Davis 1985), which may potentiate the response of spinal motor neurons to afferent stimulation (White and Neuman 1980). Local, selective depletion of spinal norepinephrine blocks the excitatory effect of yohimbine (Kehne and Davis 1985). An increase in norepinephrine release by yohimbine would also be expected to increase the response of facial motor neurons to afferent stimulation (McCall and Aghajanian 1979). This effect should facilitate the eyeblink component of startle which is mediated by facial motor neurons.

This investigation was not designed to directly examine the relationship between yohimbine-induced increases in startle and anxiety. For technical reasons, the startle measurements were done 80 min following drug administration, approximately 60 min after the significant effects of yohimbine on anxiety ratings. Thus, the significant correlation between yohimbine effects on startle and anxiety represents an association in magnitude but not time. These two effects of yohimbine, therefore, cannot be stated to be causally related to each other based upon the current study. The significant positive correlation probably reflects the effects of yohimbine on different sites in the central nervous system (i.e., for anxiety the locus coeruleus, amygdala, and cortical noradrenergic projections; for startle the facial motor nucleus and spinal cord).

In laboratory animals, yohimbine increases the magnitude of fear-potentiated startle (Davis et al. 1979). Investigation of this effect in humans may prove to be a method capable of more definitively assessing the relationships among noradrenergic function, anxiety, and startle responsiveness. This work could lead to a better understanding of the pathophysiology of anxiety disorders, such as post-traumatic stress disorder, characterized by exaggerated startle responses.

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