Blockade of autoreceptor-mediated inhibition of norepinephrine release by atipamezole is maintained after chronic reuptake inhibition

M. Danet Lapiz1, Zaorui Zhao2,3, Corina O. Bondi1, James M. O’Donnell2 and David A. Morilak1

1 Department of Pharmacology and Center for Biomedical Neuroscience, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA
2 Department of Behavioral Medicine & Psychiatry, and 3 Graduate Program in Pharmaceutical and Pharmacological Sciences, West Virginia University Health Science Center, Morgantown, WV, USA

Abstract

The role of α2-adrenergic autoreceptor desensitization in the delayed onset of antidepressant efficacy of selective norepinephrine (NE) reuptake inhibitors is unclear. Using the α2-antagonist yohimbine, we showed previously that chronic treatment with desipramine (DMI) did not alter autoreceptor-mediated inhibition of NE release in the cortex. However, yohimbine may have non-selective effects that could confound this interpretation. Thus, using microdialysis, we measured acute effects of the highly selective α2-antagonist atipamezole on NE release in the prefrontal cortex following chronic DMI treatment, after 0–8 d washout. Atipamezole induced a similar elevation of extracellular NE in all treatment groups, indicating no change in autoreceptor function. Further, the effect was most rapid in DMI-treated rats with 0- and 2-d washout, suggesting that autoreceptor-mediated inhibition was most prominent when NE levels were highest. This provides further evidence that autoreceptor-mediated inhibition of NE neurotransmission remains functional after chronic DMI treatment, arguing against the hypothesis that desensitization of α2-autoreceptors accounts for the delayed onset of action of selective NE reuptake inhibitors.

Received 1 November 2006; Reviewed 6 December 2006; Revised 13 December 2006; Accepted 12 January 2007; First published online 15 August 2007

Key words: Antidepressant, atipamezole, autoreceptor, desipramine, norepinephrine.

Introduction

Many antidepressants have potent pharmacological effects on central noradrenergic (NA) and/or serotonergic (5-HT) systems. Despite advances in antidepressant therapy, the mechanisms by which these drugs produce their effects are still not fully understood. Studies using selective serotonin reuptake inhibitors (SSRIs) have shown that a gradual desensitization of somatodendritic 5-HT1A autoreceptors following chronic treatment (Blier and de Montigny, 1994, 1999; Puñeyro and Blier, 1999) may contribute to a sustained and substantial elevation of extracellular serotonin in forebrain regions innervated by 5-HT terminals (Artigas et al., 2001). Although it has often been assumed that the same process would occur in the NA system, the data have been much less clear or consistent on the degree to which, or even whether α2-adrenergic autoreceptors are desensitized after selective norepinephrine (NE) reuptake blockade. There is evidence that chronic NE reuptake blockade may induce some degree of desensitization of α2-adrenergic receptors (Sacchetti et al., 2001), and attenuate the inhibitory influence of the exogenous α2-receptor agonist clonidine (Svensson, 1980). However, it has also been shown that α2-adrenergic autoreceptors remain largely functional after chronic NE reuptake blockade. For instance, tonic electrical activity of NA neurons remains inhibited following chronic NE reuptake blockade (Béique et al., 2000;
Figure 1. Effects of acute systemic administration of atipamezole on absolute extracellular norepinephrine (NE) levels expressed as pg/sample (mean ± S.E.M.), in microdialysate samples (30 min per sample) collected from the mPFC of rats chronically treated for 14 d with desipramine (DMI) followed by varying washout times after pump removal. (a) Vehicle-treated rats;
Linérr et al., 1999; Szabo and Blier, 2001; Szabo et al., 2000). We also showed recently that the acute elevation of extracellular NE levels in the medial prefrontal cortex (mPFC) induced by acute administration of the α₂-receptor antagonist, yohimbine, was not altered in rats chronically treated with the selective NE reuptake inhibitor desipramine (DMI), suggesting that the endogenous activation of α₂-autoreceptors persists in restraining NA neurotransmission in the face of tonically elevated NE levels following chronic reuptake blockade (Garcia et al., 2004). However, yohimbine has potential non-selective effects, such as partial agonist activity at serotonergic 5-HT₁A receptors (Newman-Tancredi et al., 1998), that may also alter NA transmission. Thus, in this study, we used the highly selective and potent α₂-adrenergic autoreceptor antagonist, atipamezole (Haapalinna et al., 1998). Further, we extended the previous observation by comparing the response to acute atipamezole challenge following chronic DMI treatment with 0–8 d drug washout, to assess any possible influence that the presence of the reuptake blocker may have had on the effects of α₂-autoreceptor blockade.

Methods

Animals

Sixty-nine adult male Sprague–Dawley rats were initially group-housed (three per cage), maintained on a 12-h light/dark cycle (lights on at 07:00 hours), and given access to food and water ad libitum. Rats were acclimatized for a minimum of 7 d before any procedures. Experiments were conducted during the light portion of the cycle, between 09:00 and 17:00 hours. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and were consistent with NIH guidelines for the care and use of laboratory animals. All efforts were made to minimize animal pain, discomfort or suffering and the number of rats used.

Procedures

Rats were randomly assigned to five chronic treatment groups (n = 12–17 per group): vehicle or DMI, administered by osmotic minipumps, with four different washout times following the 14-d DMI treatment (0, 2, 5 or 8 d after pump removal). Vehicle-treated rats also had pumps removed and were tested after 0, 2, 5 or 8 d washout, but as there were no differences between these vehicle groups on any measure, they were combined into a single control group. On the testing day, each chronic treatment group was further subdivided into two acute drug treatment groups, saline or atipamezole.

Rats weighing 220–250 g at the time of surgery were anaesthetized with a cocktail of 43 mg/ml ketamine, 1.4 mg/ml acepromazine, 8.6 mg/ml xylazine; 1.0 ml/kg i.m., with 25% supplement as needed. Osmotic minipumps (2ML2, Alzet Corp., Palo Alto, CA, USA), preloaded with either vehicle (10% ethanol) or DMI (Sigma, St Louis, MO, USA) at a concentration calculated to deliver 15 mg/kg.d of the free base, were implanted subcutaneously on the lateral dorsum behind the shoulder. After minipump implantation, rats were placed in a stereotaxic frame with the incisor bar set at −3.3 mm, adjusted to achieve a flat skull, indicated by equal DV coordinates for bregma and lambda. A guide cannula (CMA/12; CMA Microdialysis, North Chelmsford, MA, USA), aimed at the mPFC, was implanted using a 10° lateral approach with the following coordinates relative to bregma: AP +2.6 mm, ML +1.4 mm, DV −1.7 mm, based on the atlas of Paxinos and Watson (Paxinos and Watson, 1998). Approximately half the rats in each group were implanted on the left side, and half on the right. The guide cannula was anchored to the skull with jeweller’s screws and dental acrylic, and an obdurator was inserted to maintain patency. After surgery, rats were given an antibiotic (penicillin G, 300 000 IU/ml, 1.0 ml/kg s.c.), hydrated with 1 ml saline (i.p.), returned to a fresh cage and housed singly until testing day. After 14-d drug treatment, rats in the washout groups were anaesthetized briefly with isoflurane and the minipumps removed. The wounds were sutured, a systemic antibiotic administered, and the rats were returned to their home cages for the designated number of washout days before testing. Pumps were not removed from rats tested with no washout.

(b) chronic DMI treatment with no washout; (c) chronic DMI treatment with 2-d washout; (d) chronic DMI treatment with 5-d washout; (e) chronic DMI treatment with 8-d washout. Atipamezole significantly increased extracellular NE levels in the mPFC of all groups. * p < 0.05 compared to pre-injection baseline; + p < 0.05 compared to control rats in the same chronic treatment condition injected acutely with saline.
On the testing day, the obdurator was replaced by a microdialysis probe (CMA/12), with molecular weight cut-off of 20 kDa and 4 mm of active membrane, inserted into the guide cannula. The probe extended 4 mm beyond the tip of the guide, centring the active membrane within the mPFC. The probe was perfused with artificial cerebrospinal fluid (147 mM NaCl, 2.5 mM KCl, 1.3 mMCaCl\textsubscript{2}, 0.9 mM MgCl\textsubscript{2}; pH 7.4) at a flow rate of 1.0 \( \mu \)l/min. Animals were allowed a 2 h equilibration period before sample collection was initiated. Collection time was 30 min, resulting in a sample volume of 30 \( \mu \)l, collected into a tube containing 2.5 \( \mu \)l of stabilizing solution (0.2 \( \mu \)M EDTA, 0.2 \( \mu \)M ascorbic acid, 0.2 \( \mu \)M perchloric acid). Following collection of four baseline samples in the home cage, the rat was injected with either saline (1 ml/kg i.p.) or atipamezole (5 mg/kg), and returned to its home cage. Five samples were collected post-injection. NE was measured in dialysate samples by high-performance liquid chromatography with coulometric detection (Coulochem2, ESA Inc., East Chelmsford, MA, USA). The mobile phase contained 60 mM sodium phosphate, 75 \( \mu \)M EDTA, 1.5 mM sodium 1-octanesulfonic acid, 6\% methanol, pH 4.6, at a flow rate of 0.6 ml/min. Under these conditions, NE had a retention time of \( \approx \)7.8 min. The amount of NE in each sample was quantified against a calibration curve run daily, ranging from 0.0 to 25 pg, with a detection limit of \( \approx \)0.5 pg/sample.

**Data analyses**

All data were expressed as mean ± S.E.M. To first test for changes in tonic baseline NE levels induced in the mPFC by chronic DMI treatment with different washout periods compared to vehicle, the mean of the four baseline samples was calculated for each animal, and these values were subjected to one-way analysis of variance (ANOVA). The effect on NE release of acute \( \alpha_2 \)-adrenergic autoreceptor blockade by atipamezole administration was then analysed by three-way ANOVA with repeated measures, with two grouping factors (Chronic Treatment and Acute Drug), and one within-subject factor (Sample Time). The same analysis was conducted both for absolute NE levels (expressed as pg/sample) and with all values converted to percent of mean baseline for each rat, to allow a comparison of the relative effects of atipamezole after normalizing for any baseline differences. Where these analyses indicated significant main treatment effects and interactions, data were further analysed by two-way repeated measures ANOVA comparing effects of acute atipamezole and saline injections in each chronic treatment group (vehicle and DMI, with each washout time). Where significant effects or interactions were indicated in either analysis, post-hoc comparisons were performed using the Newman–Keuls test. Significance was determined in all analyses at \( p < 0.05 \).

At the end of each experiment, rats were sacrificed by rapid decapitation. Trunk blood was collected for analysis of plasma DMI levels (assays by Dr M. Javors, Department of Psychiatry, UTHSCSA), and brains removed for histological verification of probe placement. All probe tracks were found to be localized to the mPFC.

**Results**

Chronic 14-d DMI treatment, with no washout, resulted in mean plasma DMI levels of 255.1 ± 44.2 ng/ml. By contrast, DMI levels were undetectable (<5 ng/ml) in all vehicle-treated groups and in all washout groups, including 2, 5 and 8 d.

Analysis of mean baseline NE levels indicated a significant treatment effect \( (F_{4,16} = 48.20, p < 0.001) \). Post-hoc analyses indicated that extracellular NE levels in DMI-treated rats with no washout were significantly higher than all other groups (Figure 1).

Analysis of absolute NE levels following acute saline or atipamezole injection showed significant effects of Chronic Treatment \( (F_{4,16} = 113.04, p < 0.001) \), Acute Drug \( (F_{1,16} = 101.70, p < 0.001) \) and Sample Time \( (F_{4,16} = 14.62, p < 0.001) \), with a significant interaction between the three variables \( (F_{4,16} = 8.72, p < 0.001) \).

Subsequent two-way ANOVA showed significant effects of Acute Drug and Sample Time in each chronic treatment condition, and post-hoc analyses showed that for all treatment groups, atipamezole significantly increased NE levels in the mPFC compared both to pre-injection baseline and to the corresponding acute vehicle-injected group (all \( p < 0.05 \); Figure 1).

Because of the significant difference in baseline NE levels after chronic DMI treatment, data were also analysed after normalizing to percent of mean baseline. This of course obviated any main effect of Chronic Treatment \( (F_{4,16} = 1.78, p = 0.14) \), but as above, there were significant main effects of Acute Drug \( (F_{1,16} = 68.34, p < 0.001) \) and Time \( (F_{4,16} = 11.41, p < 0.001) \), and a significant interaction between the three variables \( (F_{4,16} = 2.01, p < 0.05) \). Also as above, two-way ANOVA for each chronic treatment condition, followed by post-hoc analyses, showed that atipamezole treatment significantly increased NE levels in the mPFC compared both to pre-injection baseline and to the corresponding vehicle-injected group (all \( p < 0.05 \), Figure 2).
Figure 2. Effects of acute systemic administration of atipamezole on extracellular norepinephrine (NE) levels expressed as percent of mean baseline (mean ± S.E.M.), in microdialysate samples (30 min per sample) collected from the mPFC of rats chronically treated for 14 d with desipramine (DMI) followed by varying washout times after pump removal. (a) Vehicle-treated rats; (b) chronic DMI treatment with no washout; (c) chronic DMI treatment with 2-d washout; (d) chronic DMI treatment with 5-d washout; (e) chronic DMI treatment with 8-d washout. Atipamezole significantly increased extracellular NE levels in the mPFC of all groups. *p < 0.05 compared to pre-injection baseline; + p < 0.05 compared to control rats in the same chronic treatment condition injected acutely with saline.
Discussion

This study provides further evidence, using the highly selective and potent α2-adrenergic receptor antagonist atipamezole, that autoreceptor-mediated inhibition of NA neurotransmission in the mPFC remains largely functional after chronic DMI treatment. Systemic administration of atipamezole induced a similar acute elevation of extracellular NE levels in all treatment groups. Interestingly, although we did not explicitly analyse the time-course of acute response to atipamezole in the different treatment groups, the response seemed, if anything, most rapid in DMI-treated rats with no washout and in the 2-d washout group, suggesting that autoreceptor-mediated inhibition was most prominent when extracellular levels of NE were at their highest, further evidence that autoreceptors remained functional after chronic reuptake blockade. By 5 d washout, when extracellular NE levels had returned completely to baseline, the response to atipamezole was similar to that in rats treated chronically with vehicle.

A possible explanation for the discrepancy between our results and others that have reported α2-adrenergic autoreceptor desensitization may derive from the fact that many of these previous studies relied on agonist administration to probe autoreceptor sensitivity, and baseline levels of autoreceptor activation may change as a result of the elevated extracellular NE levels after chronic reuptake blockade. Thus, even if autoreceptors remain fully functional after chronic reuptake blockade, acute application of an exogenous agonist may appear to have relatively less effect because of the higher degree of competition at the autoreceptor exerted by the endogenous neurotransmitter.

These results support a growing body of evidence that, contrary to the desensitization of 5-HT1A autoreceptors observed following chronic serotonin reuptake inhibition, α2-adrenergic autoreceptors remain functional after chronic NE reuptake blockade, arguing against the hypothesis that α2-autoreceptor desensitization may account for the delayed onset of clinical efficacy of selective NE reuptake inhibitors. Other mechanisms have been suggested that may account for the delayed clinical effect following chronic monoamine reuptake blockade, for instance down-regulation of the NE or serotonin transporter (Bennmansour et al., 2002, 2004). It is interesting to note that the time-course required for recovery of serotonin transporter expression and function following cessation of chronic serotonin reuptake blockade (Bennmansour et al., 2002) was consistent with the 2–5 d time-course required for full recovery of tonic extracellular NE levels in the mPFC following pump removal in the present study, even though DMI was undetectable in plasma after only 2 d washout. By contrast, persistence of α2-adrenergic autoreceptor function in attenuating NE release and the excitability of the NA system could account for other relevant effects, such as the anxiolytic efficacy of NE-selective antidepressant drugs (Versiani et al., 2002).

Acknowledgements

Expert technical assistance was provided by Ms. Selinda Salazar. Support was provided by research grants from the National Institutes of Health (MH72672 and MH40694).

Statement of Interest

None.

References


