Clinical doses of atomoxetine significantly occupy both norepinephrine and serotonin transports: Implications on treatment of depression and ADHD

Y.-S. Ding a,d,⁎, M. Naganawa a, J.-D. Gallezo a, N. Nabulsi a, S.-F. Lin a, J. Ropchan a, D. Weinzierla, T.J. McCarthy b, R.E. Carson a, Y. Huang a, M. Laruelle c

a Department of Diagnostic Radiology, Yale University, New Haven, CT, USA
b Pfizer Global RD, Groton, CT, USA
c New Medicines, UCB Pharma S.A., Brussels, Belgium
d Department of Psychiatry and Radiology, New York University, New York, NY, USA

A R T I C L E   I N F O

Article history:
Accepted 1 August 2013
Available online 9 August 2013

Keywords:
Atomoxetine
Attention deficit hyperactivity disorder
Depression
Norepinephrine transporter
Serotonin transporter
Positron emission tomography

A B S T R A C T

Background: Atomoxetine (ATX), a drug for treatment of depression and ADHD, has a high affinity for the norepinephrine transporter (NET); however, our previous study showed it had a blocking effect similar to fluoxetine on binding of [11C]DASB, a selective serotonin transporter (SERT) ligand. Whether the therapeutic effects of ATX are due to inhibition of either or both transporters is not known. Here we report our comparative PET imaging studies with [11C]MRB (a NET ligand) and [11C]AFM (a SERT ligand) to evaluate in vivo IC50 values of ATX in monkeys.

Methods: Rhesus monkeys were scanned up to four times with each tracer with up to four doses of ATX. ATX or saline (placebo) infusion began 2 h before each PET scan, lasting until the end of the 2-h scan. The final infusion rates were 0.01–0.12 mg/kg/h and 0.045–1.054 mg/kg/h for the NET and SERT studies, respectively. ATX plasma levels and metabolite-corrected arterial input functions were measured. Distribution volumes (VT) and IC50 values were estimated.

Results: ATX displayed dose-dependent occupancy on both NET and SERT, with a higher occupancy on NET: IC50 of 31 ± 10 and 99 ± 21 ng/mL plasma for NET and SERT, respectively. At a clinically relevant dose (1.0 ± 0.6 mg/kg, approx. 300–600 ng/mL plasma), ATX would occupy >90% of NET and >85% of SERT. This extrapolation assumes comparable free fraction of ATX in humans and non-human primates.

Conclusion: Our data suggests that ATX at clinically relevant doses greatly occupies both NET and SERT. Thus, therapeutic modes of ATX action for treatment of depression and ADHD may be more complex than selective blockade of NET.

© 2013 Published by Elsevier Inc.

Introduction

Atomoxetine (ATX, Strattera) is the first non-stimulant drug that was approved for the treatment of attention-deficit/hyperactivity disorder (ADHD) (Arnsten, 2006; Bymaster et al., 2002; Chamberlain et al., 2007a; Del Campo et al., 2011; Michelson et al., 2001; Swanson et al., 2006; Volkow et al., 2001). It has also been used to improve responses to treatment of depression (Dell’Osso et al., 2010; O’Sullivan et al., 2009). The majority of medications currently used in the treatment of ADHD or depression act to increase brain catecholamine levels; e.g., dopamine (DA), norepinephrine (NE) and serotonin. However, the precise mechanisms that are responsible for the therapeutic effects of these medications remain elusive. For example, based on the current literature, the therapeutic effects of methylphenidate, the most common treatment for ADHD, are due mostly to its dopamine transporter (DAT) inhibition. However, our recent in vivo PET imaging study demonstrated, for the first time in humans, that oral methylphenidate significantly occupies norepinephrine transporter (NET) at clinically relevant doses with an ED50 of 0.14 mg/kg, which is lower than that for DAT (0.25 mg/kg), suggesting the potential relevance of NET inhibition in the therapeutic effects of methylphenidate in ADHD (Hannestad et al., 2010).

ATX is another effective drug for the treatment of ADHD, belonging to a different structural class than methylphenidate (being a distant relative of tricyclics). It is thought that ATX acts via blockade of the presynaptic norepinephrine transporter (NET) in the brain; however, our previous study showed that it had a blocking effect similar to fluoxetine (a selective serotonin transporter [SERT] inhibitor) on binding of [11C]DASB (Ding and Fowler, 2005), a selective SERT ligand with a Ki value of 1.77 nM (Houle et al., 2000). These results suggested that ATX not only binds to NET, but also binds to SERT with high affinity. To date, whether the therapeutic effects of ATX in the treatment of either ADHD or depression are due to inhibitory effects on one (NET) or two transporters (NET and SERT) – see front matter © 2013 Published by Elsevier Inc.

http://dx.doi.org/10.1016/j.neuroimage.2013.08.001

1051-1195; see front matter © 2013 Published by Elsevier Inc.
and SERT) is not known. The aim of this investigation was to carry out comparative occupancy studies with \(^{11}\)C-MIB \(\text{(a NET ligand)}\) (Ding et al., 2003, 2005, 2006); and \(^{11}\)C-AFM \(\{[^{11}\text{C}]2-\text{(dimethylaminomethylphenylthio)}]-5\text{-fluoromethylphenylamine, a SERT ligand with a Ki value of 0.42 nM}\} (Huang et al., 2002) to evaluate the in vivo IC\(_{50}\) of ATX for both NET and SERT in non-human primates (NHP) using positron emission tomography (PET) imaging.

In order to maintain a constant concentration of ATX in the plasma during the PET acquisition time, a continuous infusion protocol was used for both occupancy studies. The detailed evaluation and discussion on the suitability of \(^{11}\)C-MIB for drug occupancy studies of NET with ATX has been published (Gallezot et al., 2011). We report here the detailed evaluation of the SERT occupancy study with ATX using \(^{11}\)C-AFM. A direct comparison of the outcome of these two studies and their potential implication on the therapeutic mechanisms of ATX will also be discussed.

Materials and methods

Study design

The continuous infusion protocol was similar to our previous study with \(^{11}\)C-MIB (Gallezot et al., 2011). Two infusion rates were used: 2 h before the beginning of each post-ATX PET scan, the first ATX infusion at the "loading" rate was begun; then, 1 h before the injection of \(^{11}\)C-AFM and the beginning of the PET scan, the infusion rate was lowered by 37.5% \(\text{(i.e., "maintenance" rate). This infusion rate was maintained until the end of PET scan. Two monkeys were scanned with maintenance rates: 0.045 mg/kg/h \(\text{(low dose), 0.135 mg/kg/h \(\text{(medium dose), and 0.405 mg/kg/h \(\text{(high dose). One monkey was scanned with the higher maintenance rates: 0.135 mg/kg/h \(\text{(medium dose), 0.405 mg/kg/h \(\text{(high dose), and 1.054 mg/kg/h \(\text{(very high dose). The design was to have each monkey to be scanned for a total of four times on two study days. These two study days were scheduled at least three weeks apart to allow recovery from the effect of the anesthesia and blood drawing. On each study day, two PET scans were acquired, beginning 4 h apart. The first study day consisted of the baseline and medium-dose scans, the second study day consisted of the low- and high-dose scans.}\)

Study population

The study protocol was approved by the Yale University Institutional Animal Care and Use Committee. Three monkeys were scanned. One monkey was scanned four times at the higher maintenance rates. Due to the unavailability of arterial blood sampling, the other monkeys were scanned twice \(\text{(baseline and low-dose for one, baseline and medium-dose for another). The monkeys ranged in age from four to eight years old and body weight from 6.2 to 9.1 kg.}\)

Radiochemistry

\(^{11}\)C-AFM was prepared by N-methylation of the precursor with \(^{11}\)C methyl triflate using the PETrace cyclotron, Mel Microlab®, and TRACERlab™ FXC automated synthesizer \(\text{(GE Healthcare, Chalfont St Giles, UK). Briefly, \(^{11}\)C-methyl triflate was trapped in 400 μL anhydrous methyl ethyl ketone containing 0.5–1 mg precursor at 0 °C, and the resulting mixture was heated for 1 min at 60 °C, and then purified by RP-HPLC. The PET drug was isolated by solid phase extraction and formulated into 9% ethanolic saline, then passed through a 0.22 μm membrane filter for final sterilization. The average chemical and radiochemical purities were >99.9% and 93.5 ± 3.5% \((n = 8). The average injected dose was 250 ± 17 MBq with average injected mass of 0.81 ± 0.45 μg.}\)

PET imaging

Animals were initially anesthetized with a ketamine hydrochloride \(\text{(10 mg/kg, IM)}, and then transported to the PET suite for scan preparation at least 2 h prior to the initial PET scan. Once intubated, the animals were maintained on oxygen and isoflurane \(\text{(1.5%)}\). Scans were performed with the High Resolution Research Tomograph \(\text{(HRRT)}\) \(\text{(Siemens/CTI Knoxville, TN, USA)}\) PET scanner which acquires 207 simultaneous slices with 1.2 mm inter-slice distance and resolution of ~3 mm. Following a 6-min transmission scan, \(^{11}\)C-AFM was injected over 2 min by an infusion pump \(\text{(Harvard PHD 22/2000, Harvard Apparatus, Holliston, Massachusetts, USA)}\). List-mode data were acquired for 120 min. Dynamic scan data were reconstructed with all corrections (attenuation, normalization, scatter, randoms, and deadtime) using the MOLAR algorithm \(\text{(Carson et al., 2003; Johnson et al., 2004). A sequence of 33 frames was reconstructed with the following timing: 6 × 30 s, 3 × 1 min, 2 × 2 min, and 22 × 5 min.}\)

Arterial input function measurement

In order to measure the time–concentration curve of \(^{11}\)C-AFM, 21 blood samples were drawn at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, and 120 min after the injection of \(^{11}\)C-AFM. Sample volumes were approximately 0.5 mL. Samples drawn at 3, 6, 15, 30, 60, and 90 min postinjection had a volume of 1.5 mL in order to measure the fraction of unchanged \(^{11}\)C-AFM in plasma by HPLC. Plasma was separated from blood cells by centrifugation \(\text{(3900 g for 5 min at 4 °C; Allegra X-22R Centrifuge, Beckman Coulter, Fullerton, CA, USA)}\). Whole blood and plasma samples were counted in a cross-calibrated well counter \(\text{(Wizard 1480, Perkin Elmer, Waltham, MA, USA)}\). Plasma proteins were precipitated using equal amounts of acetonitrile and centrifugation at 14,000 g for 5 min. The supernatant was counted and 0.2 mL was analyzed by reverse-phase HPLC with a Luna C18(2) column \(\text{(semi-prep, 10 × 250 mm, 10 μm, Phenomenex, Torrance, CA, USA)}\). The column was eluted with a mixture of acetonitrile and aqueous 0.1 M ammonium formate pH 4.2 \(\text{(32/68)}\) at a flow rate of 4.3 mL/min. The parent fraction was calculated as the ratio of the total radioactivity in fractions containing the parent compound to the total radioactivity collected, and fitted to a sum of exponentials \((f_p)\). In addition, the time-varying extraction efficiency of radioactivity in acetonitrile was measured from the counts in the supernatant and the pellet. The extraction fractions were fitted to a polynomial curve \((f_e)\). The final unchanged tracer fraction in plasma was then computed as the product of the functions \(f_p\) and \(f_e\).

\(^{11}\)C-AFM plasma free fraction

The procedure for measurement of plasma free fraction \((f_p)\) of \(^{11}\)C-AFM was identical to that used in our previous study with \(^{11}\)C-MIB (Gallezot et al., 2011). Briefly, for measurement of tracer plasma free fraction \((f_p)\), ~740 kBq of \(^{11}\)C-AFM was added \(~25 \mu\text{L/mL blood from the dose vial) to a blood sample withdrawn before injection to produce a blood standard. After mixing and centrifugation of red blood cells, plasma filtrate was separated from plasma proteins using ultrafiltration tubes \(\text{(Centriprep UF device number 4104, Millipore, Billerica, MA, USA)}\) and centrifugation \(\text{(1100 g for 20 min; EC Medilite Centrifuge, Thermo Fisher Scientific, Waltham, MA, USA)}\). Plasma and filtrate samples were counted in triplicate and free fraction \((f_p)\) was estimated as the ratio of the mean concentrations in filtrate and plasma.

ATX plasma levels

In addition to the samples used to determine \(^{11}\)C-AFM radioactivity concentration in plasma, four 3-mL blood samples were drawn 60, 120, 180, and 240 min post-ATX infusion to determine the ATX concentration in plasma. The plasma level of ATX was quantified with capillary
gas chromatography–mass spectrometry (Analytical Psychopharmacology Laboratory, Nathan Kline Institute, NY, USA).

**Magnetic resonance imaging and regions of interest**

T1-weighted MR images were acquired for each rhesus monkey on a Siemens 3 T Trio scanner and co-registered with PET images. In order to compute regional time activity curves, selected regions of interest (ROIs) where drawn on a template brain (a representative MR image of a monkey brain) for the brainstem (1.85 cm³), thalamus (0.25 cm³), globus pallidus (0.12 cm³), insula (0.96 cm³), putamen (0.38 cm³), caudate nucleus (0.43 cm³), hippocampus (0.32 cm³), amygdala (0.097 cm³), temporal lobe (3.18 cm³), anterior cingulate cortex (0.077 cm³), cingulate cortex (0.64 cm³), occipital lobe (5.22 cm³), frontal lobe (2.49 cm³), and cerebellum white matter (0.51 cm³), and cerebellum (5.91 cm³). To transfer the template of regions to the MR image of each monkey, an affine 12-parameter coregistration matrix was estimated using a normalized mutual information algorithm (FLIRT).

**Estimation of [11C]AFM volume of distribution and ATX IC\textsubscript{50} and ED\textsubscript{50}**

Although the cerebellum is accepted as reference region for SERT studies, existence of some specific binding in the cerebellum has been reported (Hinz et al., 2008; Kish et al., 2005; Parsey et al., 2006; Szabo et al., 2002). Given the higher affinity of AFM than DASB, we chose not to compute binding potential. Instead, we used the occupancy plot, which was originally introduced by Lassen et al. (1995), and subsequently by Cunningham et al. (2010). The occupancy plot uses regional estimates of volume of distribution (\(V_i\)), and can be used in the absence of a reference region. Regional \(V_i\) values of [11C]AFM were estimated using the multilinear analysis method (MA1) (Ichise et al., 2002).

When MA1 was applied to the regional time activity curves, \(t^*\) was set to 40 min after administration. Standard errors of \(V_i\) and \(V_i/\rho\) were estimated using the error propagation equation. Using the volume of distribution corrected for the plasma free fractions (\(V_i/\rho\)) transporter occupancy (Occ) was calculated as the slope of the occupancy plot from the following equation:

\[
\frac{V_i}{\rho} \text{(post-drug)} - \frac{V_i}{\rho} \text{(baseline)} = \text{Occ} \left( \frac{V_i}{\rho} \text{(baseline)} - \frac{V_{ND}}{\rho} \right).
\]

Then, ATX IC\textsubscript{50} was estimated by fitting the ATX occupancy values obtained from multiple experiments versus the ATX plasma concentration (\(C_{\text{ATX}}\)) using the following equation:

\[
\text{Occ} = \frac{C_{\text{ATX}}}{C_{\text{ATX}} + \text{IC}_{50}}
\]

The IC\textsubscript{50} estimates [ng/mL plasma] were converted to ED\textsubscript{50} estimates [ng/kg/h] using the linear correlation between the measured ATX plasma levels and the infusion rates.

**Results**

**ATX plasma levels**

For all monkey studies, ATX plasma levels could be considered stable 120 min after the beginning of the first infusion for all doses (low, medium, high and very high doses), ranging between 50 ng/mL and 1132 ng/mL (Fig. 1A). Moreover, average ATX plasma levels and the infusion rate were well correlated (Fig. 1B). The slope of the regression line was 1.12 kg x h/L (\(r^2 = 0.96, n = 5\), intercept was set to zero).

![Fig. 1](image)

**[11C]AFM input function**

The fraction of unmetabolized [11C]AFM in plasma decreased rapidly (Fig. 2A) with parent fractions of 38 ± 5% and 8 ± 5% at 6 and 30 min post-injection, respectively. [11C]AFM plasma free fraction was reasonably stable from scan to scan (Fig. 2B) (22% ± 4%, \(n = 8\)) and larger than that reported previously in baboons (9.6 ± 0.5%, \(n = 4\), Huang et al., 2002).

**[11C]AFM distribution volumes**

Fig. 3 shows typical regional time-activity curves and fits to the MA1 model for a baseline scan with \(t^*\) set to 40 min. The distribution volume (\(V_i\)) values of [11C]AFM are shown in Table 1. In baseline scans, regional brain [11C]AFM distribution volume reflected the known distribution of SERT, with high binding in the brainstem and thalamus, intermediate binding in neocortex, and lowest binding in the cerebellum. Parametric images of \(V_i\) from monkey #3 are shown in Fig. 4, providing visualization of dose-dependent occupancy. \(V_i\) values were reduced throughout the brain at all doses (Fig. 5). In particular, \(V_i\) in the cerebellum was reduced by 48% (monkey #1), 29% (monkey #2), and 22 ± 8% (monkey #3), suggesting that there was some contribution of specific binding to the cerebellum signal.

**Estimating ATX IC\textsubscript{50} using occupancy plot**

Occupancy plots with the normalized distribution volumes are shown in Figs. 5A–C. ATX occupancy was estimated for each scan as the slope of the regression line. The ATX occupancy estimates were 46% for the low dose scan for monkey #1 (Fig. 6A), 58% for the medium dose scan for monkey #2 (Fig. 6B), and 64%, 79%, and 92% for the medium-, high-, and very high-dose scans for monkey #3 (Fig. 6C). The relationship between the ATX occupancy estimates and the measured concentration...
of ATX in plasma is shown in Fig. 6D. The ATX IC\textsubscript{50} computed with the normalized distribution volume was estimated to be 99 ng/mL (relative standard error (rSE) = 21%) of plasma ATX concentration, which corresponds to an infusion rate of 0.088 mg/kg/h. If the calculation was performed without normalization by \( f_p \), the ATX IC\textsubscript{50} estimate was very similar, 87 ng/mL, but with higher uncertainty (rSE = 41%).

**Summary of the comparative occupancy studies of ATX for NET (using \([11C]\)MRB) and SERT (using \([11C]\)AFM)**

ATX displayed dose-dependent occupancy of both NET and SERT, with a higher occupancy of NET: IC\textsubscript{50} of 31 ± 10 and 99 ± 21 ng/mL plasma for NET and SERT, respectively (Gallezot et al., 2011). At a clinically relevant dose (1.0–1.8 mg/kg, approx. 300–600 ng/mL plasma), ATX would occupy >90% of NET and >85% of SERT. This extrapolation assumes comparable free fraction of ATX in humans and NHP.

**Discussion**

**Occupancy analysis**

In our previous study, variability in the \( V_T \) estimates was observed using \([11C]\)MRB post-ATX (Gallezot et al., 2011). A dose-dependent reduction was found when the \( V_T \) was normalized with free fraction (\( V_T/f_p \)). Normalization of \( V_T \) with \( f_p \) should, in principle, reduce variability in \( V_T \) if variability in \( f_p \) is substantial and if it is measured accurately. In this study using \([11C]\)AFM with ATX, we could see a dose-dependent reduction using either \( V_T \) or \( V_T/f_p \). However, the fitting of the occupancy curve (Fig. 6D) and the precision of the ATX IC\textsubscript{50} estimate were improved using the normalized distribution volume. Therefore, \( V_T/f_p \) was used as the primary outcome parameter in this study as well.

**Selectivity of ATX**

Earlier literature indicated that ATX is an equally potent inhibitor of both NET and SERT. Based on the in vitro data previously shown, (R)-\([3H]\)tomoxetine (a.k.a. atomoxetine) bound not only to the NET but also to the SERT with an affinity (Kd) of 8.9 and 2 nM for human SERT and NET, respectively (Tatsumi et al., 1997). These results were consistent with our previous study that showed that ATX had a blocking effect similar to fluoxetine (a selective serotonin transporter [SERT] inhibitor) on binding of \([11C]\)DASB (Ding and Fowler, 2005). Our comparative occupancy studies with \([11C]\)MRB (Gallezot et al., 2011) and \([11C]\)AFM (current study) to evaluate the in vivo IC\textsubscript{50} of ATX for both NET and SERT using PET in NHP further corroborate the selectivity of ATX.

**Comparison of binding affinity estimates in vivo versus in vitro**

Based on our studies, ATX displayed IC\textsubscript{50} of 31 ± 10 and 99 ± 21 ng/mL plasma for NET and SERT, respectively. It is worth noting that this value of 3.2 for the selectivity ratio of ATX towards NET vs. SERT measured via in vivo PET imaging in NHP is close in magnitude to the selectivity ratio of 4.5 derived from the previously reported in vitro Kd values using human NET and SERT tissue homogenates (Tatsumi et al., 1997).

<table>
<thead>
<tr>
<th>Region</th>
<th>Monkey</th>
<th>Distribution volume ( V_T ) [mL/cm\textsuperscript{3}]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum #1</td>
<td></td>
<td>42.1</td>
</tr>
<tr>
<td>Frontal lobe #1</td>
<td></td>
<td>47.2</td>
</tr>
<tr>
<td>Caudate #1</td>
<td></td>
<td>118.9</td>
</tr>
<tr>
<td>Thalamus #1</td>
<td></td>
<td>111.8</td>
</tr>
<tr>
<td>Brainstem #1</td>
<td></td>
<td>162.4</td>
</tr>
<tr>
<td><strong>ATX low dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum #2</td>
<td></td>
<td>37.2</td>
</tr>
<tr>
<td>Frontal lobe #2</td>
<td></td>
<td>76.3</td>
</tr>
<tr>
<td>Caudate #2</td>
<td></td>
<td>74.2</td>
</tr>
<tr>
<td>Thalamus #2</td>
<td></td>
<td>129.4</td>
</tr>
<tr>
<td>Brainstem #2</td>
<td></td>
<td>141.1</td>
</tr>
<tr>
<td><strong>ATX medium dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum #3</td>
<td></td>
<td>36.3</td>
</tr>
<tr>
<td>Frontal lobe #3</td>
<td></td>
<td>45.2</td>
</tr>
<tr>
<td>Caudate #3</td>
<td></td>
<td>74.2</td>
</tr>
<tr>
<td>Thalamus #3</td>
<td></td>
<td>142.3</td>
</tr>
<tr>
<td>Brainstem #3</td>
<td></td>
<td>141.1</td>
</tr>
<tr>
<td><strong>ATX high dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum #4</td>
<td></td>
<td>21.9</td>
</tr>
<tr>
<td>Frontal lobe #4</td>
<td></td>
<td>53.8</td>
</tr>
<tr>
<td>Caudate #4</td>
<td></td>
<td>45.5</td>
</tr>
<tr>
<td>Thalamus #4</td>
<td></td>
<td>67.1</td>
</tr>
<tr>
<td>Brainstem #4</td>
<td></td>
<td>86.5</td>
</tr>
<tr>
<td><strong>ATX very high dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum #5</td>
<td></td>
<td>26.2</td>
</tr>
<tr>
<td>Frontal lobe #5</td>
<td></td>
<td>45.5</td>
</tr>
<tr>
<td>Caudate #5</td>
<td></td>
<td>49.9</td>
</tr>
<tr>
<td>Thalamus #5</td>
<td></td>
<td>52.1</td>
</tr>
<tr>
<td>Brainstem #5</td>
<td></td>
<td>56.9</td>
</tr>
</tbody>
</table>

**Table 1**

\([11C]\)AFM distribution volumes \( (V_T) \).
are CYP2D6 poor metabolizers (PM); i.e., most are extensive metabolizers (EM). The primary oxidative metabolite of atomoxetine is 4-hydroxyatomoxetine, which is subsequently conjugated to form the corresponding glucuronidate. Although differences were observed in the excretion and relative amounts of metabolites formed, the primary difference observed between EM (extensive metabolizer) and PM (poor metabolizer) subjects was the rate at which atomoxetine was biotransformed to 4-hydroxyatomoxetine. Within 24 h postdose of [14C]-atomoxetine, ~90% of the total radioactive dose had been excreted by EM subjects, whereas only 27% of the total dose was excreted by PM subjects (Sauer et al., 2003). It has also been shown that atomoxetine was highly bound to plasma protein. The plasma protein binding of total radioactivity at 2 h after administration of [14C]-atomoxetine in EM subjects and PM subjects was 54% and 97%, respectively, suggesting that the net free fraction of total C-14 in plasma (a combination of ATX and metabolites) varies with metabolism, and ATX has much higher protein binding than metabolites. The metabolic profile, the extent of metabolism rate and the free fraction of ATX in NHP have not been well characterized. Thus, it is difficult to relate the in vivo ATX IC50 plasma concentration in NHP (31 ng/mL, 106 nM for NET; or 99 ng/mL, 339 nM for SERT) with the previously reported Kd values for human in vitro (2 nM and 8.9 nM for NET and SERT, respectively). Furthermore, the difference between in vivo and in vitro Kd might be due to error in free fraction in plasma (but also are potentially sensitive to free fractions of the two PET tracers in brain), gradient across the blood–brain barrier or assay conditions, etc. Interestingly, if NHP are poor metabolizers with the 3% free fraction, the in vivo IC50 measurement would be a perfect match with the in vitro affinity (i.e., 3 nM = 106 × 0.03 for NET affinity; or 9 nM = 339 nM × 0.03 for SERT affinity).

Noradrenergic action in ADHD and depression

ADHD is a major psychiatric disorder in children, but effective treatments remain elusive and the neurochemical mechanisms of this disorder are poorly understood. It has been widely accepted that the cognitive and behavioral impairment characteristics of ADHD are caused by the dysregulation in dopamine (DA) and norepinephrine (NE) circuits (American Psychiatric Press, 1994; Barkley, 1997; Dopheide and Pliszka, 2009). The role of NE in ADHD is supported by animal studies showing that many of the symptoms of ADHD can be recreated by blocking α-2 NE receptors in the prefrontal cortex (PFC) (Arnsten, 2009; Chappell et al., 1995; Horrigan and Barnhill, 1995; Hunt et al., 1995; Ma et al.,
The most compelling evidence supporting the role of DA and NE in ADHD comes from treatment studies. Medications used in the treatment of ADHD (including methylphenidate, dextroamphetamine and atomoxetine) act to increase brain DA and NE. For example, the most widely prescribed pharmacological treatment for patients with ADHD has been methylphenidate (MP, Ritalin). Though most studies have focused on its effects on the dopamine system, we have recently demonstrated that oral MP significantly occupies NET at clinically relevant doses with an ED50 of 0.14 mg/kg for NET, lower than that for DAT (0.25 mg/kg), suggesting the role of NET inhibition in the therapeutic effects of drugs for treatment of ADHD (Hannestad et al., 2010).

The noradrenergic action also exerts an important clinical effect in different antidepressant classes such as desipramine and nortriptiline (tricyclics, prevalent noradrenergic effect), reboxetine and atomoxetine (relatively pure noradrenergic reuptake inhibitors (NRIs)), and dual action antidepressants such as the serotonin noradrenaline reuptake inhibitors (SNRIs), the noradrenergic and dopaminergic reuptake inhibitor (NDRI) bupropion, and other compounds (e.g., mianserin, mirtazapine), which enhance the noradrenergic transmission. Even some atypical antipsychotics, such as quetiapine, and its metabolite norquetiapine in particular, act on the noradrenergic system binding the NET.

The development of ATX and its approval for the treatment of ADHD by the Food and Drug Administration (Faraone et al., 2005) were based on the rationale that it selectively blocks the NET (Barton, 2005). The deficits of inhibition control (a.k.a. impulsivity) can be measured by the stop-signal task (SST), and quantified by the stop-signal reaction time (SSRT), an estimate of the time taken to inhibit the prepotent motor response (Aron et al., 2003). The research group at Cambridge, UK found that single doses of atomoxetine (60 mg) were able to improve response inhibition in humans and in rats (Bari et al., 2009; Chamberlain et al., 2006, 2007a; Eagle et al., 2008). By contrast, serotonin manipulations with citalopram, a SERT blocker (Chamberlain et al., 2006, Del Campo et al., 2011), or with Buspirone, a serotonin 5-HT1A receptor (Chamberlain et al., 2007b) appear to have no behavioral effects on the SSRT. These results suggest that atomoxetine enhances stopping selectively via actions on NE uptake, and that NET drugs may be more suited to ameliorating impaired response inhibition as an ADHD therapeutic target.

Taken together, the inhibitory effect of ATX on NET plays a crucial role on its therapeutic effect on ADHD, while the inhibitory effect of ATX on SERT may play a more important role on its therapeutic effect on depression. Further studies to elucidate the actual relationship between the drug mechanisms and drug therapeutic effects are needed.
Acknowledgment

The authors thank the staff of the Yale University PET Center for their technical expertise and support, and Tom Cooper at Nathan S. Kline Institute for Psychiatric Research (Orangeburg, New York) for measuring blood levels of atomoxetine for this study. Funding for the ATX-NET study and the ATX-SERT study was provided by the Yale Pfizer and Yale GSK Bioimaging Alliance, respectively.

Conflict of interest

Marc Laruelle is a full-time employee of UCB Pharma, and a GSK shareholder. Timothy McCarthy is a full time employee of Pfi zer. None of the other authors reports any biomedical financial interests or potential conflicts of interests.

References


Arnsten, A.F., 2009. Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. CNS Drugs 23 (Suppl. 1), 33–41.


