The pro-psychotic metabotropic glutamate receptor compounds fenobam and AZD9272 share binding sites with monoamine oxidase-B inhibitors in humans

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HIGHLIGHTS

- mGluR5 ligands fenobam and AZD9272 have been reported to bind at unknown sites.
- Binding of [11C]AZD9272 is correlated with localization of MAO-B in human brain.
- Fenobam or MAO-B compounds inhibit binding of radiolabeled AZD9272.
- AZD9272 or fenobam inhibit binding of MAO-B radioligand [11C]-deprenyl-D2.
- Binding to MAO-B may possibly explain some adverse events of these compounds.

ARTICLE INFO

Keywords:
PET imaging
Autoradiography
Metabotropic glutamate receptor 5
Monoamine oxidase-B
Fenobam
AZD9272

ABSTRACT

The metabotropic glutamate receptor 5 (mGluR5) ligands fenobam and AZD9272 have been reported to induce psychosis-like adverse events and to bind at unknown, non-GluR5-related, sites. Based on similarities of the regional binding patterns for [11C]AZD9272 and the monoamine oxidase-B (MAO-B) radioligand [11C]-deprenyl-D2 in PET studies of the human brain we tested the hypothesis that the unique binding of fenobam and AZD9272 may represent specific binding to the MAO-B. PET data previously acquired for subjects examined using [11C]AZD9272 or [11C]-deprenyl-D2 were re-evaluated to assess the correlations between radioligand binding parameters in human brain. In addition, the pharmacology of AZD9272 binding sites was characterized using competition binding studies carried out in vivo in non-human primates (NHPs) and in vitro using autoradiography in selected human brain regions. The regional binding of [11C]AZD9272 in human brain was closely correlated with that of [11C]-deprenyl-D2. In PET studies of NHP brain administration of the MAO-B ligand L-deprenyl inhibited binding of radiolabeled AZD9272 and administration of fenobam inhibited binding of [11C]-deprenyl-D2. Binding of radiolabeled AZD9272 in vitro was potently inhibited by fenobam or MAO-B compounds, and [11C]-deprenyl-D2 binding was inhibited by fenobam or AZD9272. The findings are consistent with the hypothesis that both fenobam and AZD9272 bind to the MAO-B, which may be of relevance for understanding the mechanism of the psychosis-like adverse events reported for these compounds. Such understanding may serve as a lead to generate new models for the pathophysiology of psychosis.
1. Introduction

The metabotropic glutamate receptor subtype 5 (mGluR5) is a target for drug development and for molecular imaging of the glutamate system in neurological and psychiatric disorders (Pillai and Tipre, 2016). Modulation of mGluR5 activity has been suggested as a mechanism for the treatment of several conditions, such as pain, depression, anxiety and levodopa-induced dyskinesia (Emmitte, 2013). For such purposes, a number of non-competitive mGluR5 antagonists (negative allosteric modulators; NAMs) have been developed, of which the majority are structural analogues of the prototype mGluR5 NAM MPEP (Gasparini et al., 1999). Fenobam, a compound developed by McNeil Laboratories (Porter et al., 2005) and AZD9272 developed by Astrazeneca (Raboisson et al., 2012; Fig. 1) are structurally distinct, non-alkyne-containing, mGluR5 NAMs having reached clinical research. However, both compounds were discontinued due to observations of psychotic symptoms in early clinical trials (Friedmann et al., 1980; Pecknold et al., 1982; Ståhle et al., 2012). Interestingly, in contrast to fenobam and AZD9272, other mGluR5 NAM drug candidates have shown favorable safety profiles and reached more advanced stages of clinical evaluation (Quiroz et al., 2016).

Imaging of brain mGluR5 can be achieved using positron emission tomography (PET) employing mGluR5-selective radioligands. Of the mGluR5 PET radioligands hitherto developed, \([^{11}C]\)ABP688 (Ametamey et al., 2007) and \([^{18}F]\)FPEB (Sullivan et al., 2013; Wong et al., 2013) have been the most widely applied in human studies. The drug candidate mentioned above, AZD9272, has also been radiolabeled with carbon-11 and evaluated as a potential mGluR5 radioligand in non-human primates (NHPs) and human subjects (Kågedal et al., 2012; Andersson et al., 2013; Varnäs et al., 2018). Importantly, an unexpected observation during development of the radioligand \([^{11}C]\)AZD9272 was that the regional binding pattern appeared to be different from that of \([^{11}C]\)ABP688 and other mGluR5 radioligands. Moreover, while the binding of \([^{11}C]\)AZD9272 in NHP brain could be inhibited by fenobam, the binding could only partly be displaced by MPEP-like mGluR5 NAMs (Varnäs et al., 2018) suggesting that fenobam and AZD9272 recognize additional, potentially non-mGluR5-related binding sites. Insight into the nature of this binding is of interest for understanding the pharmacology of fenobam and AZD9272 in humans and to identify a potential pro-psychotic mechanism.

In line with the above, visual inspection of PET parametric images (Fig. 2A–C) reveals that while the mGluR5-specific radioligand \([^{11}C]\)ABP688 displays relatively similar binding across cortex and striatum with lower binding in other regions, \([^{11}C]\)AZD9272 shows highest binding in striatum, thalamus and brainstem. These brain regions have a high density of the monoamine oxidase-B (MAO-B) enzyme (Saura et al., 1992; Tong et al., 2013). Indeed, visual comparison of parametric images for \([^{11}C]\)AZD9272 and, more recently, the images obtained at our laboratory using the MAO-B radioligand \([^{11}C]\)L-deprenyl-D2 (Arakawa et al., 2017) shows strikingly similar distribution patterns in the human brain (Fig. 2B and C). These observations may suggest that the signal obtained with \([^{11}C]\)AZD9272 to some degree reflects binding to the MAO-B.

The objective of the present study was to test the hypothesis that the unique binding of fenobam and AZD9272 may represent specific binding to MAO-B. First, the correlation between the regional cerebral binding of \([^{11}C]\)AZD9272 and \([^{11}C]\)L-deprenyl-D2 was assessed based on evaluation of PET data previously acquired in human subjects.

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Fig. 1. Molecular structures of ABP688 (Hintermann et al., 2007), AZD9272 (Raboisson et al., 2012), fenobam (Porter et al., 2005), lazabemide (Henriot et al., 1994), L-deprenyl (Knoll and Magyar, 1972), MPEP (Gasparini et al., 1999), MTEP (Cosford et al., 2003) and SL25.1188 (Hicks et al., 2015).
Second, the pharmacology of fenobam and AZD9272 binding sites was characterized by competition binding experiments carried out in vivo in PET studies of NHP, and in vitro, in tissue sections of selected human brain regions.

2. Material and methods

2.1. Compounds

\([18\text{F}]\text{AZD9272}\) (molar activity, \(\text{MA} > 10\ \text{GBq}/\mu\text{mol}) was produced from its nitro precursor in a one-step fluorine-18 nucleophilic substitution reaction (Nag et al., 2018). \([11\text{C}]\text{-L-deprenyl-D2}\) (\(\text{MA} > 195\ \text{GBq}/\mu\text{mol}) was produced as previously described elsewhere (Sturm et al., 2017). \([3\text{H}]\text{AZD9272}\) (\(\text{MA}, 0.51\ \text{GBq}/\mu\text{mol}) was synthesized at AstraZeneca, Mölndal, Sweden as described in the supplementary material. Fenobam, AZD9272, and MTEP (Cosford et al., 2003; Fig. 1) were kindly provided by AstraZeneca and SL25.1188 (Hicks et al., 2015) was kindly provided by Dr. Kenneth Dahl, Harvard Medical School, Boston, MA, USA. Other compounds and chemicals were obtained from commercially available sources and were of analytical grade.

2.2. PET studies in human subjects

Data were obtained from five previous PET studies of human control subjects examined at the Karolinska Institutet PET centre using \([12\text{C}]\text{AZD9272, [11C]}\text{ABP688 or [11C]}\text{-L-deprenyl-D2 as radioligands (Supplementary Table S1). Procedures for PET data acquisition have been described previously and parts of the data have been published (Kågedal et al., 2012, 2013; Arakawa et al., 2017). The studies were approved by the Research Ethics Committee in Stockholm, Sweden and the Radiation Safety Committee at Karolinska University Hospital, Stockholm, and were performed in accordance with the current amendment of the Declaration of Helsinki and International Conference on Harmonization/Good Clinical Practice guidelines. Written informed consent was obtained from all participants.

Ten male subjects (aged 24–38 y), had been previously examined using \([11\text{C}]\text{AZD9272, and 12 male subjects (aged 23–40 y) had been previously examined using [11C]}\text{ABP688 as a radioligand. In addition to the subjects included in the two publications by Kågedal et al. (2012, 2013; n = 6 for [11C]AZD9272 and [11C]ABP688, respectively), unpublished data acquired using the same PET methodology were in the present analysis included for 4 subjects examined using [11C]AZD9272 and 6 subjects examined using [11C]ABP688. Finally, six subjects (age range, 21–62 years; four males, two females) who had been previously examined with \([11\text{C}]}\text{-L-deprenyl-D2 were included (Arakawa et al., 2017).

Data for \([11\text{C}]}\text{ABP688 PET examinations (injected radioactivity 290–345 MBq), were acquired using the High Resolution Research Tomograph system (HRRT; Siemens Molecular Imaging, Knoxville, TN, USA) over 63 min, and data for \([11\text{C}]}\text{AZD9272 (192–318 MBq) or [11C]}\text{L}-deprenyl-D2 (315–505 MBq) examinations were acquired using the ECAT Exact HR47 system (Siemens/CTI, Knoxville, TN, USA) over 93 or 63 min, respectively.

For all subjects, individual T1-weighted brain magnetic resonance images (MRIs), acquired using a 1.5T system, were segmented into gray matter, white matter, and CSF as previously described (Arakawa et al., 2017) and co-registered with PET images. Regions of interest (ROIs) for analysis of radioligand binding were defined using the automated anatomical labelling template (Tzourio-Mazoyer et al., 2002).

Regional time activity curves for \([11\text{C}]}\text{ABP688 and [11C]}\text{AZD9272 were analyzed using the Logan non-invasive approach (Logan et al., 1996) to estimate distribution volume ratios (DVRs) for ROIs relative to the cerebellum. For both tracers, the analysis was based on data from...
63 min and the time of the linear phase (t*) was fixed at 15 min. Binding potential (BPND) was subsequently calculated based on regional estimates of DVR and volume of distribution (VT) for the cerebellum and non-displaceable volume of distribution previously reported for [18F]ABP688 (Kågedal et al., 2013) and [11C]AZD9272 (Kågedal et al., 2012). Procedures for analysis of [11C]L-deprenyl-D2 PET data, to obtain the regional parameter K3/ks k3 (k3), have been previously described by Arakawa et al. (2017). Parametric images were obtained for the purpose of visual inspection and comparison of the binding patterns for the different radioligands as described in the supplementary material.

Linear regression approaches were applied to disentangle the potential components of mGluR5 and MAO-B binding in the [11C]AZD9272 images. Initially, simple linear regression and correlation analyses were performed between the regional binding of [11C]AZD9272 and the binding of the mGluR5 and MAO-B specific radioligands, [11C]ABP688 and [11C]L-deprenyl-D2, respectively. Furthermore, in an attempt to gain a comprehensive understanding of the relative contributions of mGluR5 and MAO-B binding to the signal for [11C]AZD9272, respectively, the relationship between the binding of [11C]AZD9272 and both of these components was assessed in a combined multilinear regression analysis using regional BPND for [11C]AZD9272 (BPND, AZD) as the dependent variable and regional BPND for [11C]ABP688 (BPND, ABP) and k3 for [11C]L-deprenyl-D2 (k3, dep), respectively, as the independent variables. Specifically, the following model was fitted to the data:

\[
BP_{ND, AZD} = x_1 \times BP_{ND, ABP} + x_2 \times k_3, \text{dep} + \epsilon
\]  

(1)

### 2.3. PET studies in non-human primates

The study was approved by the Animal Ethics Committee of the Swedish Animal Welfare Agency (Dnr 145/08, 399/08 and 386/09) and was performed according to the “Guidelines for planning, conducting and documenting experimental research” (Dnr 4820/06–600) of the Karolinska Institutet and the “Guide for the Care and Use of Laboratory Animals” (Garber et al., 2011). Four male NHPs (Macaca fascicularis), weighing 5.3–7.8 kg, were supplied by the Astrid Fagreaus Laboratory, Karolinska Institutet, Solna, Sweden.

In preparation for planned studies in human subjects an 18F-labeled analogue of AZD9272 was developed. Importantly, in terms of biological and physicochemical properties the two molecules are identical. Moreover, a preliminary analysis indicates a similar pattern of radio-metabolite formation for [18F]AZD9272 and [11C]AZD9272 in NHP plasma (Nag et al., 2018; Nag et al., manuscript). PET measurements using [18F]AZD9272 were carried out in two NHPs. Each PET measurement with [18F]AZD9272 was performed on a separate experimental day. NHP #1 was examined at baseline, as well as in three subsequent experimental sessions after administration of 1.0 mg/kg of fenobam, MTEP, or L-deprenyl (Fig. 1) and in a fifth experimental session after administration of a lower dose (0.25 mg/kg) of L-deprenyl. NHP #2 was examined at baseline and in two subsequent experiments after administration of 0.25 mg/kg of fenobam or MTEP, respectively.

Experimental sessions conducted in the same NHP were separated by at least 6 weeks. In each of two additional NHPs (#3 and #4) two PET measurements with [11C]L-deprenyl-D2 were undertaken during the same experimental session, at baseline and after administration of 1.0 mg/kg of fenobam, respectively.

Based on previous findings in NHP PET studies, the highest (1.0 mg/kg) dose of fenobam and MTEP was selected to reach approx. 80% occupancy of brain mGluR5 (De Lorenzo et al., 2011; Mathews et al., 2014), whereas L-deprenyl (1.0 mg/kg) was expected to reach 70% occupancy of brain MAO-B (Nag et al., 2016).

Test compounds were administered as a 10-min intravenous infusion starting 15 min prior to the PET measurement for fenobam and MTEP and 45 min prior to the PET measurements for L-deprenyl based on previous investigations of these compounds (Nag et al., 2016; Varnäs et al., 2018). The NHP was observed continuously during the PET experimental days. Body temperature was maintained by Bair Hugger heater – Model 505 (Arizant Healthcare Inc., MN) and monitored with an esophageal thermometer. Heart and respiratory rates were continuously monitored during the experiment. The NHP head was immobilized in a head fixation system (Karlsson et al., 1993). Anesthesia was induced by intramuscular injection of ketamine hydrochloride (ca. 10 mg/kg, Ketalar®, Pfizer) and maintained by intravenous infusion of a mixture of ketamine hydrochloride (4 mg/kg/h) and xylazine hydrochloride (0.4 mg/kg/h Rompun® Vet., Bayer) for [18F]AZD9272 PET studies, and by administration of a mixture of sevoflurane, oxygen and medical air after endotracheal intubation in [11C]L-deprenyl-D2 PET studies. A sterile physiological phosphate buffer solution (pH = 7.4) of the radiotracer was injected as a bolus into a sural vein during 5 s simultaneously with the start of PET data acquisition.

PET measurements were conducted using the HRRT system. Emission data were acquired in list mode for 123 min for [18F]AZD9272 (injected radioactivity, 146–163 MBq) and 93 min for [11C]L-deprenyl-D2 (injected radioactivity, 133–167 MBq). Dynamic images were reconstructed using three-dimensional ordinary Poisson ordered subset expectation maximization including modeling of the system’s point spread function.

Arterial blood was sampled as previously described (Finnema et al., 2014) using an automated blood sampling system during the first 3 min of each PET measurement. Subsequently, arterial blood samples (0.7–3 mL) were manually drawn at 7, 15, 30, 60, 90 and 120 min after injection of [18F]AZD9272 and at 5, 15, 30, 45, 60 and 90 min after injection of [11C]L-deprenyl-D2. After centrifugation 0.2–1.5 mL plasma was pipetted and plasma radioactivity was measured in a well counter. In addition, samples were taken directly from the ABSS at 0.5, 1, 1.5, 2, 2.5 and 3 min for cross-calibration with the well counter and for determination of the plasma to blood ratio.

The fraction of plasma radioactivity corresponding to unchanged radioligand in plasma was determined from arterial plasma samples collected at 2, 7, 15, 30, 60, 90 and 120 min after injection of [18F]AZD9272 and at 2, 5, 15, 30, 45, 60 and 90 min after injection of [11C]L-deprenyl-D2 according to previously described procedures (Halldin et al., 1995). The unbound fraction of radioligand in plasma was determined using ultrafiltration as reported in the literature (Moein et al., 2019).

MRIs of the individual NHP brains had been previously obtained using a 3T General Electric Discovery MR750 (GE, Milwaukee, WI, USA) system. ROIs were manually delineated as previously described (Varnäs et al., 2018) on T1-weighted MRIs using an in-house image analysis software (Roland et al., 1994). MRIs were coregistered to averaged PET images using SPMS (Wellcome Department of Imaging Neuroscience, UK). Time-activity curves were generated by pooling ROIs for each paired anatomical region and applying the pooled ROIs to PET images using the affine transformation matrix acquired from coregistration of the MRI.

PET data were analyzed using the software PMOD v. 3.6. Regional estimates of the VT for [18F]AZD9272 were obtained based on 75 min of data acquisition using the Logan linear graphical method (Logan et al., 1990) and t* fixed at 39 min. Occupancy at [18F]AZD9272 binding sites was estimated based on regional VT values obtained at baseline and after drug administration according to a graphical method described in the literature (Cunningham et al., 2010). For [11C]L-deprenyl-D2 measurements the time activity curves were analyzed as previously described (Arakawa et al., 2017) using a two-tissue compartment model with three rate constants (K1, k2, k3) assuming irreversible binding to the second compartment. MAO-B occupancy was calculated as the percent reduction of k3 following fenobam administration relative to that obtained at baseline.
2.4.1. Brain tissue

Studies including human brain tissue were approved by the Ethics Committee at Karolinska Institutet (registration no. 03–767) and the Semmelweis University Human Ethical Committee (registration no. 113/1995, 180/2001). Human brains were obtained from clinical autopsies at the National Institute of Forensic Medicine, Karolinska Institutet (Stockholm, Sweden) and at the Department of Forensic Medicine, Semmelweis Medical University, Budapest, Hungary.

Brains were removed during autopsy and were handled in a manner similar to that described previously (Hall et al., 1998). Tissue was obtained from 4 donors (2 males and 2 females; ages 32–82 y) with no known neurological or psychiatric diagnosis at the time of death. Postmortem times were < 24 h for all cases. From visual inspection at autopsy and during sectioning, none of the brains exhibited damages or abnormalities.

Whole hemisphere tissue was cryosectioned using a heavy-duty cryomicrotome (Leica cryomacrocut CM3600, Leica, Nussloch, Germany). Cryosections with a slice thickness of 100 μm were transferred to gelatinized glass plates (10 × 22 cm), dried at room temperature and stored (−20°C) with dehydrating agents until use. In addition to the whole hemisphere sections, tissue sections from smaller blocks were used to detect binding in the brainstem, thalamus, and caudate nucleus. These frozen sections, with a thickness of 20 μm, were prepared from the tissue blocks in a cryomicrotome (Zeiss Microm, Germany). The slides were kept at −20 °C until used.

2.4.2. Autoradiography – experimental procedure

Autoradiography studies were performed according to previously described procedures (Raboisson et al., 2012; Varnäs et al., 2019). Adjacent sections were incubated with [3H]AZD9272 (3 nM) in the absence or presence of a saturating concentration (10 μM) of fenobam, the two mGlur5 NAMS MPEP and MTEP, or the MAO-B selective compounds L-deprenyl (Knoll and Magyar, 1972) and lazabemide (Henriot et al., 1994; Fig. 1). In addition, competition binding studies using [3H]AZD9272 as a radioligand were undertaken at increasing concentrations (1 nM–10 μM) of MPEP or L-deprenyl, respectively.

Regional differences in inhibitor binding were further characterized in whole hemisphere sections (100 μm) at the level of thalamus and hippocampus. Since an 18F-labeled analogue of AZD9272 having a higher molar radioactivity was developed during the course of the study (Nag et al., 2018), this radioligand was used for detailed evaluation of these tissue samples. Adjacent sections were incubated with [18F]AZD9272 (0.02 MBq/ml) in the absence or presence of a saturating 10 μM concentration of the five inhibitor substances listed above, or the MAO-B selective compound SL25.1188 (Hicks et al., 2015).

Tissue sections were pre-incubated at room temperature for 30 min in the absence or presence of test compound in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4) containing 3 mM MgCl2 and were subsequently incubated at room temperature for 60 min in the same incubation solutions containing radiolabeled AZD9272. Sections were washed (2 × 5 min) in 50 mM HEPES/3 mM MgCl2 buffer (4°C), rinsed in deionized water (4°C) and dried.

Autoradiography studies using [11C]L-deprenyl-D2 as radioligand the sections were pre-incubated for 30 min in TRIS buffer (50 mM, pH 7.4) containing NaCl (300 mM), KCl (5 mM), and ascorbic acid (0.1%, w/v) in the absence or presence of fenobam, AZD9272, MPEP or MTEP at concentrations of 100 nM or 10 μM. Sections were subsequently incubated for 20 min in the same solution containing [11C]L-deprenyl-D2 (0.2 MBq/ml) and were then washed and dried as previously described (Gulyás et al., 2011).

2.4.3. Image analysis

Radioactivity was detected and quantified using a BAS-5000 phosphor imager (Fuji, Tokyo, Japan). Autoradiographic images (pixel size = 25 × 25 μm2) were processed and quantified using the software Fiji (Schindelin et al., 2012). Image pixel values were converted into photostimulated luminescence (PSL)/mm2 units. For quantification of the binding of [3H]AZD9272 plastic tritium standards (American Radiolabeled Chemicals Inc., St. Louis, MO, USA) were used for transformation of the image intensity values into radioactivity units and binding density, expressed as nmol/g tissue. The inhibition constant corresponding to the concentration of MPEP or L-deprenyl required for half-maximum inhibition of [3H]AZD9272 binding (IC50) was estimated by nonlinear parametric curve fitting using GraphPad Prism 7.0 (GraphPad Software, Inc.). For quantification of [18F]AZD9272 and [11C]L-deprenyl-D2 binding the measured PSL/mm2 values were transformed into radioactivity units based on intensity values obtained using radioactivity standards from serial dilutions of the incubation solution.

2.5. Data and code availability statement

Due to institutional restrictions, the data for human subjects cannot be shared openly. These data are pseudonymised according to national (Swedish) and EU legislation, and cannot be anonymised and published in an open repository. The data can instead be made available upon request on a case by case basis as allowed by the legislation and ethical permits. Requests for access can be made to the Karolinska Institutet’s Research Data Office at rdo@ki.se. The data for studies not involving human subjects will be made available upon request.

3. Results

3.1. Regional radioligand binding in the human brain in vivo

Average parametric PET images for [11C]ABP688, [11C]L-deprenyl-D2 and [11C]AZD9272, are shown in Fig. 2. Visual inspection of parametric images for [11C]AZD9272 showed conspicuous differences in binding patterns in comparison to that displayed by [11C]ABP688, whereas the distribution for [11C]AZD9272 and [11C]L-deprenyl-D2 was similar in human brain (Fig. 2A–C). Radioligand binding patterns were compared on the basis of regional estimates for availability of binding sites (BPND) for [11C]AZD9272 and [11C]ABP688 as well as λD for [11C]L-deprenyl-D2. All regional parameter estimates are presented in Supplementary Table S2.

The rank order for the regional BPND values obtained for [11C]AZD9272 (striatum > amygdala > thalamus > hippocampus > pons > isocortex > cerebellum) differed from that for [11C]ABP688 (isocortex > striatum > hippocampus > amygdala > thalamus > pons > cerebellum), but was consistent with the binding pattern displayed by [11C]L-deprenyl-D2. Regional BPND values for [11C]AZD9272 did not correlate at a statistically significant level, with corresponding values for [11C]ABP688 (Pearson R² = 0.00; P = 0.979; Fig. 3A), but were strongly correlated with regional estimates of λD for [11C]L-deprenyl-D2 binding (Pearson R² = 0.95; P = 2 × 10−16; Fig. 3B).

Correspondingly, according to the two linear regressions, the regional binding of [11C]L-deprenyl-D2 was more suitable than that of [11C]ABP688 at predicting the binding of [11C]AZD9272 (Akaike information criterion, AIC, of −8.0 vs. 66.5, respectively; Fig. 3A and B). It should be noted that the residuals of the linear regression between [11C]AZD9272 and [11C]L-deprenyl-D2 binding parameters showed a moderate and significant correlation with the binding of [11C]ABP688 (Pearson R² = 0.62, P = 3 × 10−6; Supplementary Fig. S1).

An attempt was made using a multiple linear regression approach to disentangle the components of MAO-B and mGlur5 binding in the [11C]AZD9272 images. Parametric images predicting BPND for [11C]AZD9272 based on the multilinear model and [11C]ABP688 and [11C]L-deprenyl-D2.
\[1^{11}\text{C}]\text{L-deprenyl-D2} data as predictors were generated (Fig. 2D). The parametric images displayed high similarity with the corresponding average parametric images for \[1^{11}\text{C}]\text{AZD9272} (Fig. 2C). Consequently, the multilinear regression indicated that predictions of \[1^{11}\text{C}]\text{AZD9272} binding using both mGluR5-specific (\[1^{11}\text{C}]\text{ABP688}) and MAO-B-specific (\[1^{11}\text{C}]\text{L-deprenyl-D2}) components had the highest degree of correlation (Pearson \(R^2=0.98\), \(P=1\times10^{-20}\)) and best agreement (\(\text{AIC}=-29.5\)) with the observed \(\text{BP}_{\text{ND}}\) values (Fig. 3C). According to the multilinear model, roughly 15% (5–20%) of regional \(\text{BP}_{\text{ND}}\) of \[1^{11}\text{C}]\text{AZD9272} is due to mGluR5-specific binding with higher contributions in cortical areas and lower in striatum, thalamus or pons (Fig. 3C and Fig. S2). Furthermore, visual inspection of the parametric image predicting \[1^{11}\text{C}]\text{AZD9272} binding using the multilinear model on the voxel level (Fig. 2D) showed best overall similarity to the observed mean binding image (Fig. 2C), in particular with regard to the higher cortical binding levels when compared to the pattern seen in the \([1^{11}\text{C}]\text{L-deprenyl-D2}\) image (Fig. 2B).

3.2. Pharmacological characterization of fenobam and AZD9272 binding

3.2.1. In vivo characterisation in NHP brain

Altogether eight PET measurements were conducted with \([1^{18}\text{F}]\text{AZD9272}\) and four with \([1^{11}\text{C}]\text{L-deprenyl-D2}\). Due to technical problems with PET data acquisition radioactivity was measured for 102, and not 123 min, in one of the baseline \([1^{18}\text{F}]\text{AZD9272}\) measurements and for 75 min in the \([1^{18}\text{F}]\text{AZD9272}\) measurement after administration of 1.0 mg/kg MTEP. The free fraction for \([1^{18}\text{F}]\text{AZD9272}\) in plasma was similar between the two baseline measurements (0.32 and 0.35) and the six measurements post drug administration (0.20–0.34). The plasma free fraction for \([1^{11}\text{C}]\text{L-deprenyl-D2}\) was 0.19 and 0.21, respectively, for the two NHPs at baseline and remained unchanged after administration of fenobam.

The binding of \([1^{18}\text{F}]\text{AZD9272}\) in NHP brain was inhibited in a dose-dependent fashion by fenobam reaching occupancies of 53% and 84%, respectively, at the 0.25 mg/kg and 1.0 mg/kg dose levels. At both doses administered (0.25 and 1.0 mg/kg) MTEP inhibited a minor fraction (< 5%) of \([1^{18}\text{F}]\text{AZD9272}\) binding, whereas \text{L-deprenyl} inhibited a major fraction (> 85%; Fig. 4; Fig. S3). In the \([1^{11}\text{C}]\text{L-deprenyl-D2}\) PET measurements in two NHPs, administration of 1.0 mg/kg fenobam inhibited 20 and 40% of the specific binding in thalamus and 30 and 34% of the specific binding in ventral midbrain (Table S3).

3.2.2. In vitro characterisation in human brain tissue

In cryosections of the human thalamus and brainstem fenobam (10 μM) inhibited 97–99% of \([3^{3}\text{H}]\text{AZD9272}\) binding, whereas MPEP and MTEP at the same concentration induced partial inhibition of the binding (55–83% and 48–58%, respectively). The MAO-B ligands \text{L-deprenyl} or lazabemide (10 μM) inhibited 94–96% of the binding in these regions. Representative autoradiographic images of \([3^{3}\text{H}]\text{AZD9272}\) binding in the absence and presence of 10 μM of fenobam, MTEP, \text{L-deprenyl} or lazabemide are shown in Fig. 5. When incubating brain sections at increasing concentrations of inhibitor (1 nM–10 μM; Fig. 6), the binding of \([3^{3}\text{H}]\text{AZD9272}\) was more potently inhibited by \text{L-deprenyl} than by MPEP in thalamus (IC\(_{50}\) 3.7 nM vs. 1.8 μM) and substantia nigra (IC\(_{50}\) 3.7 nM vs. 4.6 μM).

Regional differences in inhibitor occupancy were assessed using \([1^{18}\text{F}]\text{AZD9272}\) at a high molar radioactivity in whole hemisphere sections of the human brain. Fenobam inhibited binding to the same extent in all brain regions, whereas no evident inhibitory effect on radioligand
binding was observed in sections co-incubated with MTEP (results not shown). MPEP potentiated inhibition binding in the cortex and CA1 region of the hippocampus, whereas parts of the signal remained in the thalamus, CA3 of the hippocampus, dentate gyrus and the ependyma of the ventricle. L-deprenyl, lazabemide, or SL25.1188 inhibited [18F]AZD9272 binding in the CA3/dentate gyrus, thalamus and ependyma of the ventricle, but displayed low occupancy in the CA1 region and cortex (Fig. S4).

The binding of [11C]L-deprenyl-D2 in human thalamus and brainstem was potently inhibited by fenobam or AZD9272 (75–80% inhibition at 100 nM and 99% inhibition at 10 μM). The binding was only partially inhibited by MPEP (16–19% at 100 nM and 93% at 10 μM) or MTEP (14–15% at 100 nM and 66–67% at 10 μM; Fig. 7).

4. Discussion

The mGluR5 NAMs fenobam and AZD9272 have been reported to have a propensity to induce psychotic symptoms in humans and have previously been found to recognize a shared, non-mGluR5-related, site in primate brain (Varnäs et al., 2018). Based on the high density of AZD9272 binding sites in brain regions expressing MAO-B, this enzyme was considered as a potential explanation for the non-target binding of fenobam and AZD9272. Major observations of the present study were that the distribution pattern for [11C]AZD9272 is strongly correlated with the binding of the MAO-B radioligand [13C]L-deprenyl-D2 in the human brain, that the binding of radiolabeled AZD9272 can be inhibited by fenobam, or by MAO-B specific compounds, and finally, that the binding of [13C]L-deprenyl-D2 can be inhibited by AZD9272 or fenobam. Taken together, these findings are consistent with the hypothesis that fenobam and AZD9272 bind to the MAO-B enzyme in the NHP and human brain.

Based on PET data acquired for human subjects the regional binding of [11C]AZD9272 was strongly correlated with the binding to MAO-B with a small contribution of mGluR5 binding (5–20% across the brain) according to a multilinear regression model using [13C]L-deprenyl-D2 and [13C]ABP688 data as predictors. This observation was corroborated by autoradiography studies showing more potent inhibition of the binding of radiolabeled AZD9272 induced by L-deprenyl than by the mGluR5-selective compounds MPEP and MTEP. The detected small relative contribution of mGluR5 binding to the signal for [11C]AZD9272 is in apparent contrast to a previous report of nanomolar affinity of AZD9272 for human mGluR5 in vitro and a pharmacological characterization of [18F]AZD9272 recognition sites in rodent brain indicating selective binding to mGluR5 (Raboisson et al., 2012). Importantly, the density of MAO-B has been shown to be lower in rat than in human brain, where the MAO-B is more abundantly expressed in comparison to neuroreceptors, such as mGluR5 (Saura et al., 1992; Patel et al., 2007; Tong et al., 2013), thus supporting the view that species differences in the relative availability of mGluR5 and MAO-B may account for these discrepancies in selectivity.

The MAO-B compound L-deprenyl inhibited the binding of radiolabeled AZD9272 in vivo in the NHP brain (87% occupancy at 1.0 mg/kg) and in vitro in human brain tissue (IC50, 3.7 nM). To further assess whether this effect may be due to competition at MAO-B the inhibition of radiolabeled AZD9272 binding was also examined by autoradiography in presence of the structurally distinct MAO-B ligands lazabemide or SL25.1188. The regional inhibition of radioligand binding induced by these compounds was consistent with that observed for L-deprenyl thus corroborating the hypothesis that L-deprenyl inhibits AZD9272 binding to the MAO-B.

The binding of radiolabeled AZD9272 in vivo was potently inhibited by fenobam (84% occupancy at 1.0 mg/kg), but was less sensitive to MTEP (< 5% occupancy at 1.0 mg/kg). MTEP and fenobam, administered at the 1 mg/kg dose level, have previously been reported to occupy 70–90% of specific binding for [13C]ABP688 at the mGluR5 in PET studies of the baboon brain (DeLorenzo et al., 2011; Mathews et al., 2014). The negligible impact of the selective mGluR5 ligand MTEP on [18F]AZD9272 binding is consistent with our previous observations with [11C]-labeled AZD9272 (Varnäs et al., 2018), but in stark contrast to the major impact on [13C]ABP688 binding (90% occupancy at 1 mg/kg; DeLorenzo et al., 2011). The occupancy induced by fenobam at AZD9272 binding sites (ID50 = 0.21 mg/kg) is of the same magnitude as that previously reported for inhibition of the binding of [13C]ABP688 (ID50 = 0.3–1.1 mg/kg; Mathews et al., 2014). Taken together, these
observations may support that the affinity of fenobam for mGluR5 and MAO-B is of the same order in vivo.

The binding of $[^{11}C]L$-deprenyl-D2 in vitro was markedly inhibited by AZD9272 and fenobam, but was less sensitive to MPEP and MTEP (Fig. 7). Observations that the binding of AZD9272 and $L$-deprenyl is mutually displacable, and the consistent rank order of compound potency (fenobam > MPEP > MTEP) for inhibiting the respective radioligand binding further support that AZD9272, $L$-deprenyl and fenobam recognize a common site.

As part of initial drug development, AZD9272 has in routine pharmacological characterization been found to be selective for mGluR5 against 134 receptors, ion channels, transporters and enzymes (Raboisson et al., 2012). However, this in vitro screening did not include MAO-B as a target. With regard to fenobam, our findings are in some contrast to the low affinity previously reported for the rat peripheral MAO-B ($K_I > 20 \mu M$; Keck et al., 2014). Species differences in fenobam binding to rat vs. primate MAO-B are not supported by our observation that fenobam inhibited the binding of $[^{11}C]L$-deprenyl-D2 also in rat brain in vitro (98% inhibition at 10μM; unpublished data). However, such discrepancies could result from the use of different radioligands, assay conditions, or be due to possible differences in ligand recognition properties of central vs. peripheral MAO-B.

The binding of $[^{11}C]L$-deprenyl-D2 in vitro was inhibited by high concentrations of the mGluR5 NAMs MPEP and MTEP (16–19% and 14–15% inhibition, respectively, at 100 nM; 93% and 66–67% inhibition, respectively, at 10μM). To our best knowledge the affinity values of MPEP and MTEP for the human MAO-B have not been reported in the literature. Nevertheless, MTEP has been reported to be more selective than MPEP for mGluR5 vs. other targets, such as the MAO-A ($IC_{50} = 30 \mu M$ and 8μM, respectively, for MTEP and MPEP; Cosford et al., 2003). The present observations of higher potency of MPEP compared to MTEP for inhibiting $[^{11}C]L$-deprenyl-D2 binding would be consistent with a higher selectivity of the latter compound also versus MAO-B. Two mGluR5 NAMs derived from the MPEP series, MRZ-8676 (IC$_{50}$ = 125 nM; Dekundy et al., 2011) and MFZ 10-7 ($K_I = 0.77 \mu M$; Keck et al., 2014), have been previously reported to interact with MAO-B at high concentrations. While our observations suggest the possibility that this feature may also be shared by MPEP and MTEP further studies using recombinant systems selectively expressing MAO-B are required to confirm binding to this enzyme.

PET data for human subjects were obtained from separate trials involving different subjects examined using different PET systems (HRRT for studies with $[^{11}C]ABP688$ vs. ECAT HR for studies with $[^{11}C]AZD9272$ or $[^{11}C]L$-deprenyl-D2). Evaluation of tracer binding patterns in separate groups of subjects examined using different imaging methodology is a limitation of the present comparative analysis. Radioligand binding patterns were compared on the basis of group average estimates for availability of binding sites assuming that regional tracer distribution is consistent across subjects. However, it cannot be excluded that the relative abundance of binding sites (i.e. mGluR5 vs. MAO-B) may differ between the groups examined. The mean age for subjects examined using $[^{11}C]ABP688$ was higher than for subjects examined in PET studies with $[^{11}C]AZD9272$ (mean, 39y; range, 21–62 y) was higher than for subjects examined in PET studies with $[^{11}C]AZD9272$ (mean, 29y; range, 24–38y). MAO-B enzyme concentration has been reported to increase with age (Fowler et al., 1980), suggesting that the proportional binding to MAO-B may be lower for the subjects examined using $[^{11}C]ABP688$ than for the subjects examined using $[^{11}C]L$-deprenyl-D2 as a radioligand. However, these differences are not expected to confound the correlation of radioligand binding parameters, since age-related increases in MAO-B availability have been reported to be similar across brain regions (Fowler et al., 1980, Fowler et al., 1997).

PET images for $[^{11}C]ABP688$ were acquired using a different system at higher anatomical resolution (HRRT, 2 mm at full width at half maximum, FWHM; Varrone et al., 2009) than images for $[^{11}C]AZD9272$ and $[^{11}C]L$-deprenyl-D2 (ECAT HR; 3.6–4.0 mm at FWHM; Wienhard et al., 1994). Nevertheless, differences in PET system resolution are unlikely to explain the higher signal for $[^{11}C]AZD9272$ than for $[^{11}C]ABP688$, since a lower resolution PET system would be expected to underestimate binding in the small brain structures labeled by AZD9272 (Schain et al., 2012). Moreover, discrepancies in regional
binding for $^{11}$C-AZD9272 and $^{11}$C-ABP688 in the brain of NHPs has been previously found in PET studies carried out using the same PET system (Varnäis et al., 2018), supporting that differences in PET system resolution cannot account for the observed variation in binding density or radioligand distribution patterns.

Psychosis-like symptoms have been reported in clinical studies of fenobam (Friedmann et al., 1980; Pecknold et al., 1982) and AZD9272 (Ståhle et al., 2012). The present observation of a shared binding site of fenobam, AZD9272 and MAO-B inhibitors raises the possibility that such psychotic symptoms are partly mediated through mechanisms involving MAO-B. There is early evidence that non-selective MAO inhibitors may precipitate psychosis (reviewed by Price and Hopkinson, 1968) involving MAO-B. There is early evidence that non-selective MAO inhibitors may precipitate psychosis (reviewed by Price and Hopkinson, 1968) involving MAO-B. Such psychotic symptoms are partly mediated through mechanisms involving MAO-B. For instance, hallucinations and paranoid delusions have been reported after use of the MAO inhibitor pargyline for treatment of hypertension (Sutnick et al., 1964) and the MAO inhibitors isoniazid and iproniazid for treatment of tuberculosis (Pleasure, 1954). Though selective MAO-B inhibitors have been less frequently reported to induce psychotic symptoms, treatment with L-deprenyl has been found to be weakly correlated with hallucinations in Parkinson’s disease (Williams and Lees, 2005). These reports in the literature of similar symptoms with MAO compounds may suggest involvement of MAO-B-related mechanisms in fenobam- and AZD9272-associated adverse events. However, it cannot be excluded that mechanisms involving both MAO-B and mGluR5 may contribute to the psychosis-like effects observed with these compounds. A more detailed understanding of MAO-B and mGluR5 in relation to psychotic symptoms may serve to generate novel pharmacological models for psychosis.

5. Conclusions

Converging evidence based on the localization and pharmacology of AZD9272 binding sites supports the hypothesis that fenobam and AZD9272 bind predominantly to the MAO-B enzyme in primate brain. Based on previous observations of psychosis-like adverse events in human subjects receiving fenobam or AZD9272 it can be suggested that MAO-B-related mechanisms may be of relevance for these effects.

Funding

This work was supported by a grant from the Swedish Research Council [grant number 2015-02398]. The PET studies of $^{11}$C-AZD9272 and $^{11}$C-ABP688 in human subjects were supported by AstraZeneca and the PET studies of $^{11}$Cl-deprenyl-D2 in human subjects were partly supported by GE Healthcare Ltd., UK.

Declaration of competing interest

Zsolt Cselényi, Peter Johnström, Lee Kingston, Charles Elmore and Lars Farde are employees of AstraZeneca. Lars Farde has served as a panel member for evaluation of the Research Programs of the Faculty of Medicine, University of Helsinki, Finland. Katarina Varnäs has received a consulting fee from AstraZeneca. The other authors declare no potential conflict of interests.

Acknowledgements

The authors thank Åsa Södergren, Siv Eriksson and other members of the PET group at the Karolinska Institutet for excellent technical assistance. We acknowledge AstraZeneca and GE Healthcare for supporting PET studies in human subjects. We also thank AstraZeneca for providing fenobam, AZD9272, and MTEP, and Kenneth Dahl from the Harvard Medical School, Boston, MA, USA, for providing SL25.1188.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2019.107809.
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