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Short communication

Post-mortem human brain autoradiography of the norepinephrine transporter using (*S*,*S*)-[^{18}F]FMeNER-D$_2$

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Abstract

The binding of the norepinephrine transporter radioligand, (*S*,*S*)-[^{18}F]FMeNER-D$_2$, to human brain post-mortem was examined in vitro by whole hemisphere autoradiography. The rank order for the density of labelling was: locus coeruleus ≫ cortex ≈ cerebellum ≈ thalamus > caudate ≈ putamen. The NET-selectivity of binding was confirmed by co-incubation with desipramine. The dual NET/SERT inhibitor duloxetine also inhibited specific binding, whereas PE2I or citalopram had no evident effect.
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Keywords: Norepinephrine transporter; Autoradiography; (*S*,*S*)-[^{18}F]FMeNER-D$_2$; Human brain

1. Introduction

In vitro or in vivo labelling of human brain norepinephrine transporters (NETs) has so far been less extensively studied in brain research than labelling of the dopamine (DAT) and serotonin (SERT) transporters (Halldin et al., 2001). In vitro autoradiographic studies of NETs in the post-mortem human brain have so far been performed using the radioligands [^{3}H]desipramine (Donnan et al., 1991), [^{3}H]mazindol (Gross-Isseroff et al., 1988) and [^{3}H]nisoxetine (Tejani-Butt et al., 1993; Klimek et al., 1997; Ordway et al., 1997). Of these radioligands, [^{3}H]nisoxetine has been the most suitable to examine NETs within the human locus coeruleus and raphe nuclei but no other regions (Tejani-Butt et al., 1993; Klimek et al., 1997; Ordway et al., 1997).

Unfortunately, in vivo imaging of NET using positron emission tomography (PET) and [^{11}C]nisoxetine or [^{11}C]desipramine has failed in animal experiments due to high non-specific labelling (Haka and Kilbourn, 1989; Schou et al., 2002). Recently however, a labelled *O*-methyl analog of reboxetine (Fig. 1), (*S*,*S*)-[^{11}C]MeNER, has been demonstrated to bind selectively to NETs in the rodent brain in vivo (Wilson et al., 2003) as well as in the non-human primate brain (Schou et al., 2003; Ding et al., 2003).

The *O*-[^{18}F]fluoromethyl di-deuterated analog of (*S*,*S*)-[^{11}C]MeNER, (*S*,*S*)-[^{18}F]FMeNER-D$_2$, has been found to bind selectively to NETs in the monkey brain in vivo with high in vitro affinity (K_i=3.1 nM; Schou et al., 2004). A problem with (*S*,*S*)-[^{11}C]MeNER for in vivo PET measurements is that the peak equilibrium of specific binding is not reached during a 93 min PET measurement. Advantages of using ^{18}F versus ^{11}C for brain imaging include a longer half-life ($t_{1/2}$=110 min vs. 20 min), allowing specific binding to reach peak equilibrium, and a lower positron energy (650 keV vs. 960 keV) that results in improved resolution.

The aim of this study was to examine and characterise the potential of (*S*,*S*)-[^{18}F]FMeNER-D$_2$ as a radioligand

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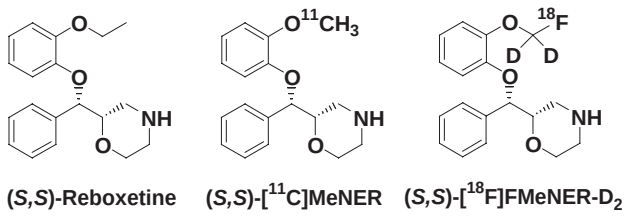

Fig. 1. Structures of (*S*,*S*)-reboxetine, (*S*,*S*)-[^{11}C]MeNER and (*S*,*S*)-[^{18}F]FMeNER-D$_2$.

for in vitro examination of NET in the human brain post-mortem.

2. Experimental procedures

2.1. Radiochemistry

(*S*,*S*)-[^{18}F]FMeNER-D$_2$ was prepared as previously described (Schou et al., 2004) giving a radiochemical purity higher than 98% and a specific radioactivity of about 148 GBq/μmol (4000 Ci/mmol).

2.2. In vitro autoradiography

Three human brains were obtained for this study from the National Institute of Forensic Medicine, Karolinska Institutet (Stockholm, Sweden). The brains (see Table 1 for details) had been removed at clinical autopsy and were handled in a manner similar to that previously described (Hall et al., 1998; Hall et al., 2001). Horizontal sections from three brain levels were examined. Level 1 was selected to contain the thalamus, caudate and putamen. Level 2 included the ventral parts of the striatum and the hypothalamus and level 3 covered the lower parts of the brain, including the locus coeruleus.

The sections were incubated for 90 min at RT with 4 MBq (*S*,*S*)-[^{18}F]FMeNER-D$_2$ in a TRIS buffer (50 mM; pH 7.4) containing sodium chloride (300 mM), potassium chloride (5 mM) and ascorbic acid (0.1% (w/v). The sections were then washed (same buffer) three times for 5 min each and briefly dipped in cold distilled water before being exposed to Kodak Biomax MR film overnight. Non-specific binding was estimated by simultaneous incubation with desipramine (10 μM). Competition studies were also performed with three further reference ligands: (i) PE2I, a selective DAT inhibitor (Page et al., 2002); (ii) citalopram, a selective SERT inhibitor (Hyttel, 1982); (iii) duloxetine, a dual NET and SERT inhibitor (Wong et al., 1993).

Specific binding was determined as total binding minus binding in the presence of excess desipramine. The specific binding was related to binding in locus coeruleus, as determined in the same experiment. The percentage specific binding to total binding was calculated for regions with more than three experiments.

Table 1
Descriptions of the brains used in the experiments

No.	Age	Sex	Post-mortem time (h)	Cause of death
001	63	Male	48	Suicide
006	32	Male	12	Cardiac death
46	33	Male	5	Cardiac infarction

3. Results

The radioactive amount of (*S*,*S*)-[^{18}F]FMeNER-D$_2$ was 4 MBq at start of incubation, which corresponded to a concentration of about 0.14 nM.

A conspicuous accumulation of radioactivity was observed in the locus coeruleus (Figs. 2 and 3; Table 2). Considerably lower binding was seen in the cerebellum, cortex, thalamus (Figs. 2, 3 and 4) or hypothalamus. The binding in these regions was abolished in cryosections incubated with desipramine, indicating specific accumulation of (*S*,*S*)-[^{18}F]FMeNER-D$_2$ to NET. Some labelling was also seen in the caudate nucleus and putamen (Fig. 4), although this labelling was not abolished by the inclusion of desipramine in the incubate. The specific binding was reduced with over 95% after co-incubation with duloxetine, resulting in similar autoradiograms as obtained in the presence of desipramine. The labelling of (*S*,*S*)-[^{18}F]FMeNER-D$_2$ to any brain region was not affected by

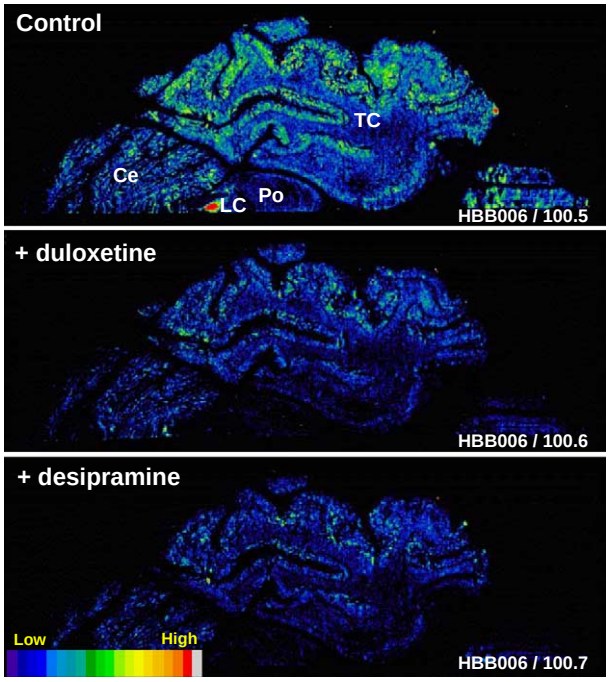

Fig. 2. Human brain autoradiograms labelled with (*S*,*S*)-[^{18}F]FMeNER-D$_2$ and with the dual NET/SERT inhibitor, duloxetine, or the selective NET inhibitor, desipramine, present in the labelling incubate solution. Brain regions included in this section are cortex and locus coeruleus.

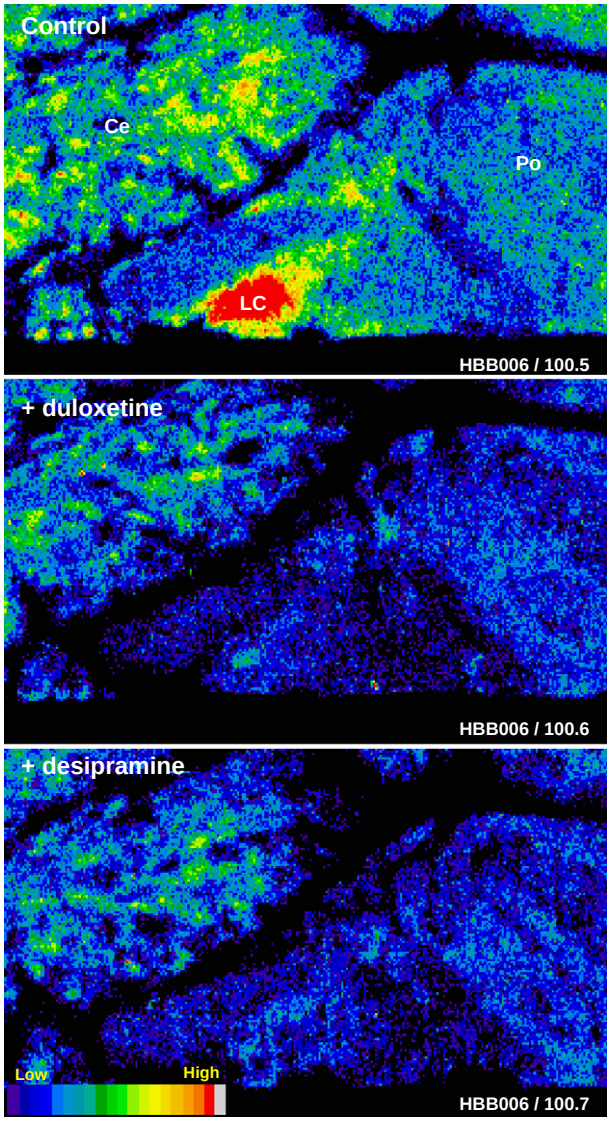

Fig. 3. Similar images as in Fig. 2, but with the region surrounding the locus coeruleus magnified.

co-incubation with the DAT inhibitor PE2I or the SERT inhibitor citalopram.

4. Discussion

Labelling of human NET outside the locus coeruleus (LC) and raphe nuclei (RN) with a selective radioligand has to the best of our knowledge not been performed previously. In vitro examination of NET in the human brain post-mortem outside the LC has so far been attempted by using either [^{3}H]mazindol (Donnan et al., 1991) or [^{3}H]desipramine (Gross-Isseroff et al., 1988). The complications with these radioligands are that [^{3}H]mazindol is a dual DAT and NET ligand and therefore requires blockade of the DAT portion of binding by co-incubation with a DAT inhibitor (Donnan et al., 1991) and that [^{3}H]desipramine in addition to the NET binding sites also binds to a second low-affinity site (Bäckström and Marcusson, 1990). [^{3}H]Nisoxetine has been proposed as a suitable radioligand for NET (Tejani-Butt, 1992; Charnay et al., 1995; Ordway et al., 1997), but the binding to NET outside the LC and RN in the human brain has not been reported. The reported regional densities of NET outside the locus coeruleus are lower than within the LC (Gross-Isseroff et al., 1988; Charnay et al., 1995). To get a reasonable signal to noise ratio, the lower densities of NETs in these regions may therefore require a selective radioligand with higher affinity and/or a lower non-specific binding than [^{3}H]nisoxetine.

Table 2
Specific binding of (S,S)-[^{18}F]FMeNER-D$_2$, in percent of binding in locus coeruleus

	Specific binding related to locus coeruleus			Specific binding/total binding		
	Percent	S.E.M.	n	Percent	S.E.M.	n
Locus coeruleus	100.0		4	47.1	7.2	4
Pons anterior	9.8	4.9	4	7.0	2.1	4
Caudatus	5.7		1			
Putamen	3.0		1			
Thalamus	8.0		1			
Temporal cortex	12.3	4.3	4	7.8	2.7	4
Insular cortex	9.9		1			
Occipital cortex	1.6		1			
Cerebellum, total	15.2	5.0	4	9.9	2.1	4
Cerebellum, grey matter	15.6	6.0	4	8.4	2.6	4
White matter	7.6	4.3	5			

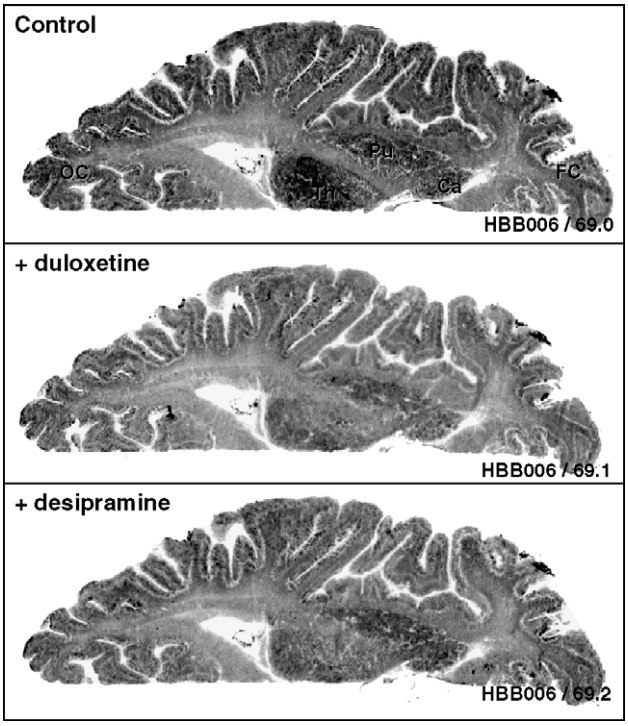

Fig. 4. Human brain autoradiograms labelled with (S,S)-[^{18}F]FMeNER-D$_2$ and with the dual NET/SERT inhibitor, duloxetine, or the selective NET inhibitor, desipramine, present in the labelling incubate solution. Brain regions included in this section are caudate, putamen and thalamus.

The present study was performed to evaluate whether (*S*,*S*)-[^{18}F]FMeNER-D$_2$ could be an alternative to [^{3}H]nisoxetine in this respect. However, one of the important prerequisites, a higher affinity, was not fulfilled. Nisoxetine is reported to have a K_i of 2.1 nM (Cheetham et al., 1996) which is similar to (*S*,*S*)-FMeNER-D$_2$ having a K_i of 3.1 nM (Schou et al., 2004). (*S*,*S*)-FMeNER-D$_2$, being less lipophilic, may however have the advantage of a lower non-specific component of total binding, since non-specific binding generally increases with lipophilicity of the radioligand.

The dense labelling of LC with (*S*,*S*)-[^{18}F]FMeNER-D$_2$ was in good agreement with the reported high density of NETs in this region (Donnan et al., 1991; Ordway et al., 1997; Gross-Isseroff et al., 1988). In other relatively NET-rich regions, like hypothalamus and thalamus, the specific binding was not sufficiently high to provide a favourable signal to noise ratio. The most likely explanation for this is that the affinity of (*S*,*S*)-[^{18}F]FMeNER-D$_2$ is insufficient to provide a robust signal in these regions with lower NET densities.

There was also some labelling of the caudate nucleus and putamen. The binding was insensitive to DAT, NET and SERT inhibition and was therefore regarded as non-specific binding. However, a similar observation has been made in PET experiments with the closely related radioligand, (*S*,*S*)-[^{11}C]MeNER (Ding et al., 2003). It cannot be excluded that this labelling represents binding to a yet unidentified specific binding site.

5. Conclusions

(*S*,*S*)-[^{18}F]FMeNER-D$_2$ is a useful radioligand for imaging of NET within the locus coeruleus of the post-mortem human brain. However, the signal to noise ratio is significantly lower outside the LC, reflecting the lower densities of NET. It therefore seems crucial to develop a selective radioligand with higher affinity for NET to enable in vitro visualization and quantification of NET in regions external to LC.

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