RADIOIODINATED ANALOGS OF XYLAMINE : N-(2-CHLOROETHYL)-N-ETHYL-2-[^{125}I]IODOBENZYLAMINE AND N,N-DIETHYL-2-[^{125}I]IODO BENZYLAMINE AS POTENTIAL TOOLS FOR MONOAMINE UPTAKE EXPLORATION BY SPECT

C. BRANGER[1], L. GARREAU[1], Y. FRANGIN[1], S. CHALON[1], P. DUBOIS[2], A.-M. DOGNON[1], J.-E. OMBETTA-GOKA[3], J.-C. BESNARD[1], D. GUILLOTEAU[1]*

[1]INSERM U316, Laboratoire de Biophysique Médicale et Pharmaceutique, Université François Rabelais, Faculté des Sciences Pharmaceutiques, 31 Avenue Monge 37200 Tours, France

[2]Laboratoire de Chimie Analytique, Université François Rabelais, Faculté des Sciences Pharmaceutiques, 31 Avenue Monge 37200 Tours, France

[3]Laboratoire de Chimie Organique Thérapeutique, Université François Rabelais, Faculté des Sciences Pharmaceutiques, 31 Avenue Monge 37200 Tours, France

* Author for correspondence

Tel: (33) 47 36 72 18 - Fax: (33) 47 36 72 24

SUMMARY

In order to improve the scintigraphy and radiotherapy of neuroendocrine tumors we synthesized two radioiodinated benzylamines [N-(2-chloroethyl)-N-ethyl-2-[^{125}I]iodobenzylamine and N,N-diethyl-2-[^{125}I]iodobenzylamine], analogs of xylamine [N-(2-chloroethyl)-N-ethyl-2-methylbenzylamine]. Xylamine is an irreversible inhibitor of uptake and accumulation of noradrenaline. The two unlabelled iodinated derivatives [N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine and N,N-diethyl-2-iodobenzylamine] were synthesized, purified and checked by HPLC, NMR and mass spectrography. Their affinity for the noradrenaline transporter was determined in vitro on rat brain membrane homogenates with [^{3}H]nisoxetine. Radioiodination was performed by iodide for bromide nucleophilic exchange from brominated precursors. The N,N-diethyl-2-[^{125}I]iodobenzylamine was

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obtained directly from N,N-diethyl-2-bromobenzylamine. Radiosynthesis of N-(2-chloroethyl)-N-ethyl-2-[^{125}I]iodobenzylamine required three steps. A new brominated precursor [N-ethyl-N-(2-bromobenzyl)glycine ethyl ester] which was stable for radiolabelling and suitable for reduction to N-(2-hydroxyethyl)-N-ethyl-2-[^{125}I]iodobenzylamine was synthesized. N-(2-Hydroxyethyl)-N-ethyl-2-[^{125}I]iodobenzylamine was converted to N-(2-chloroethyl)-N-ethyl-2-[^{125}I]iodobenzylamine in the presence of an excess of thionyl chloride. Radioiodinated derivatives were purified and checked by HPLC.

KEY WORDS : monoamine uptake, xylamine, radioiodide

INTRODUCTION

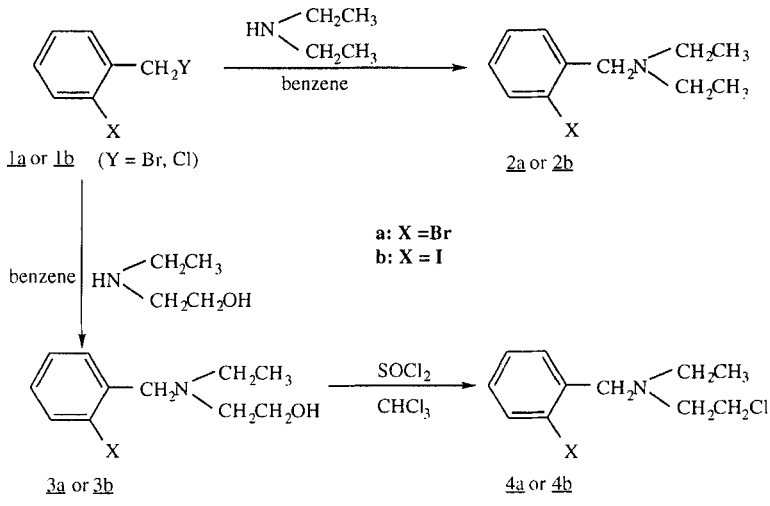
During embryogenesis some cells migrate from the neural crest and colonize different tissues. Several tumors derived from these cells have been observed. Neural crest - derived tumors such as pheochromocytoma, paraganglioma, neuroblastoma and carcinoid and medullary thyroid carcinomas (MTC) arising from these cells show some identical properties. In particular these tumors are able to take up monoamine precursors and decarboxylate them to give biogenic monoamines. For the diagnosis and therapy of these tumors we can use radiopharmaceuticals which can be concentrated in the cells by the uptake mechanism. Metaiodobenzylguanidine (mIBG) was introduced by Wieland et al. in 1980 (1), and this radiopharmaceutical is now widely used (2, 3). However mIBG does not allow detection of every neural crest-derived tumor. For example sensitivity for the detection of carcinoid and MTC is only 70% and 35% respectively (2). Moreover the results with [^{131}I]mIBG therapy are poor and very few remissions have been observed (2). We therefore hope to improve results in diagnosis and therapy by using other radiopharmaceuticals exhibiting different specificities for monoamine carriers and acting by other mechanisms, for example by covalent binding. In order to achieve this we chose to develop a new radiotracer derived from xylamine. Xylamine [N-(2-chloroethyl)-N-ethyl-2-methylbenzylamine] is a selective inhibitor of the uptake and accumulation of noradrenaline (4, 5, 6). Moreover xylamine is an irreversible inhibitor of noradrenaline uptake by PC12 pheochromocytoma cells (7). The xylamine structure is close to mIBG and bretylium structures which are both acting on the noradrenergic system. A halogenated analog of xylamine, DSP 4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine], has also been described as an irreversible inhibitor of noradrenaline uptake (8, 9, 10). We emphasize that two structures, xylamine and DSP 4 with methyl

and bromine respectively in position 2 on the phenyl ring, are both active. We can therefore hypothesize that another halogenated derivative such as iodinated derivative in position 2 on the phenyl ring would be also active - iodine has the same steric hindrance as the methyl group. The irreversible inhibition of uptake seems to be due to alkylation of the transporter by the aziridinium derivative of xylamine and DSP 4 (4, 5, 12). Such a structure which is unable to form the aziridinium derivative, would be able to bind the carrier in a reversible way and would allow an accumulation in the cells from neural crest tumors. To test this second hypothesis we developed a non-chlorinated analog of xylamine, N,N-diethyl-2-iodobenzylamine. The two unlabelled iodinated derivatives [N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine and N,N-diethyl-2-iodobenzylamine] were synthesized, purified and checked by HPLC, NMR and mass spectrography. Their affinity for the noradrenaline transporter was determined in vitro on rat brain membrane homogenates with [^{3}H]nisoxetine. Radiolabelling of the two iodinated analogs of xylamine [N-(2-chloroethyl)-N-ethyl-2-[^{125}I]iodobenzylamine and N,N-diethyl-2-[^{125}I]iodobenzylamine] was developed in order to perform future in vivo studies.

RESULTS AND DISCUSSION

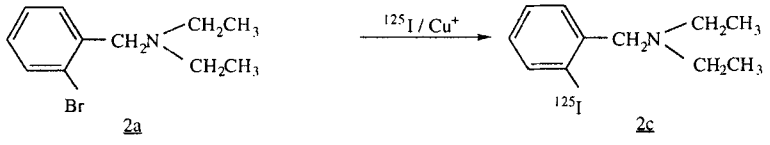
The 2-halogenobenzylamines were synthesized according to Kammerer *et al.* (3) with some modifications. N,N-Diethyl-2-bromobenzylamine (2a) or N,N-diethyl-2-iodobenzylamine (2b) was obtained in one pot synthesis from the appropriate 2-bromobenzyl bromide (1a) or 2-iodobenzyl chloride (1b) and diethylamine with 99% and 95% yields respectively (Scheme 1). N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine (4a) or N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b) was obtained in two steps (Scheme 1). The appropriate 2-bromobenzyl bromide (1a) or 2-iodobenzyl chloride (1b) was first condensed with 2-(ethylamino)ethanol to give benzylamino alcohol (3a or 3b) which then reacted with an excess of thionyl chloride to give 2-chloroethylbenzylamine (4a or 4b) with 87% and 89% yields respectively. The products were recrystallized from their salts, each compound was checked by HPLC and identified by mass and ^{1}H-NMR spectrometries.

The two iodinated benzylamines [N,N-diethyl-2-iodobenzylamine (2b) and N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b)] were tested in vitro on rat brain membrane homogenates with [^{3}H]nisoxetine. Their affinity for the noradrenaline transporter was determined: IC_{50} of N,N-diethyl-2-iodobenzylamine (2b) was >1 μM and IC_{50} of N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b) was 800 ± 60 nM. In the same experimental conditions IC_{50} of mIBG and mazindol were 500 ± 60



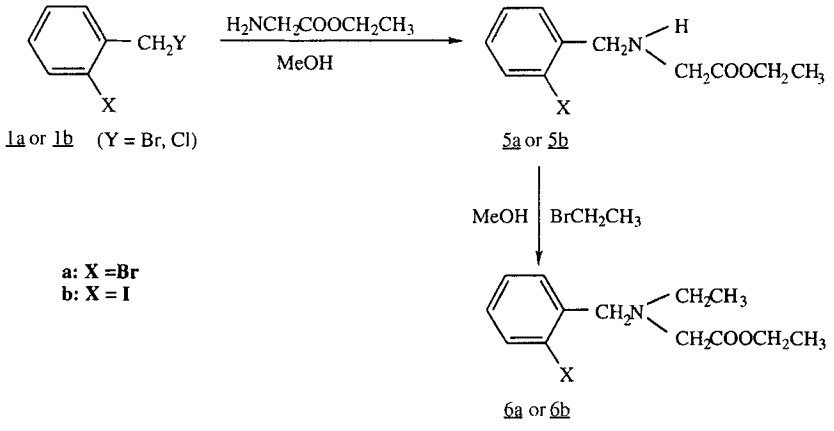
Scheme 1

nM and 15 ± 5 nM respectively. Mazindol is known to be a strong inhibitor of noradrenaline uptake (11). The IC_{50} value for N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b) (800 nM) was in the same order as the IC_{50} value for mIBG (500 nM). In view of these results N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b) could be a good tool for scintigraphic exploration. For future in vivo studies we therefore developed the two radioiodinated benzylamines [N,N-diethyl-2-[^{125}I]iodobenzylamine (2c) and N-(2-chloroethyl)-N-ethyl-2-[^{125}I]iodobenzylamine (4c)]. Radiolabelling was performed by iodide for bromide nucleophilic exchange as described by Mertens (12). This method was the most convenient to substitute [^{125}I]iodide in ortho position. By this method N,N-diethyl-2-[^{125}I]iodobenzylamine (2c) was easily obtained from N,N-diethyl-2-bromobenzylamine (2a) (Scheme 2).



Scheme 2

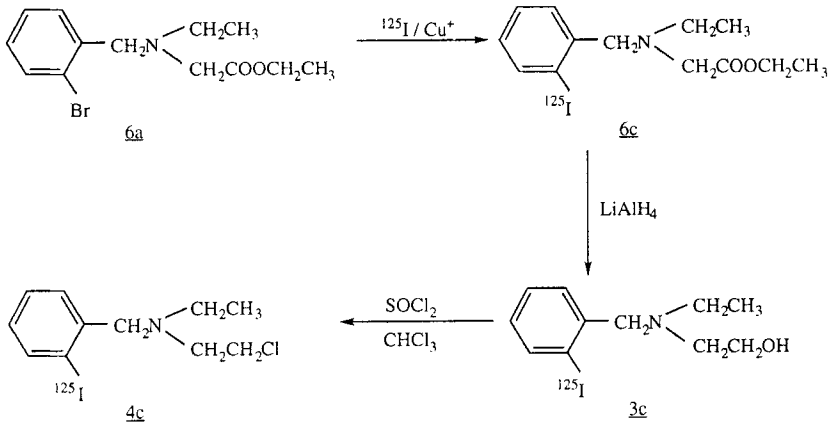
The product was purified by HPLC and identified by coinjection with the unlabelled iodinated derivative N,N-diethyl-2-iodobenzylamine (2b). For the N-(2-chloroethyl)-N-ethyl-2-[^{125}I]iodobenzylamine it was not possible to perform the radiosynthesis directly from N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (4a) or from N-(2-hydroxyethyl)-N-ethyl-2-bromobenzylamine (3a). In both these cases the radiolabelling yielded only unknown labelled by-products. These results could be expected, since the 2-chloroethyl and 2-hydroxyethyl chains are not stable in radiolabelling conditions (140°C, acid medium). A new brominated precursor N-ethyl-N-(2-bromobenzyl)glycine ethyl ester (6a) was therefore synthesized (Scheme 3).



Scheme 3

This precursor (6a) yielded the N-ethyl-N-(2-[^{125}I]iodobenzyl)glycine ethyl ester (6c) which was easily reduced with $LiAlH_4$ to N-(2-hydroxyethyl)-N-ethyl-2-[^{125}I]iodobenzylamine (3c) (Scheme 4).

The two radiolabelled products (6c and 3c) were checked by HPLC coinjection with the unlabelled corresponding derivatives (6b and 3b). Chlorination was performed in chloroform in the presence of an excess of thionyl chloride. The final product was identified by HPLC coinjection with N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b). The overall radioactivity yield was 12%. These results showed that iodinated (4b) and radioiodinated (4c) analogs of xylamine could be prepared in order to explore the noradrenaline carrier. Although the iodinated ligand (4b) was easy to synthesize, it was more difficult to prepare its radioiodinated analog (4c) since direct radiolabelling failed from 2-brominated precursors (3a) and (4a). The radioligand (4a) was obtained in three steps from 2-bromobenzyl glycine ethyl ester (6a). Replacement by iodine of the methyl group of xylamine



Scheme 4

characterizes the structural difference between xylamine and its iodinated analogs. In principle, this substitution by iodine from the methyl group should not modify the steric hindrance of the molecular structure. From this viewpoint these iodinated ligands must present inhibitory properties such as xylamine which exhibits similar structures and properties to those of bretylium and mIBG (fig 1). This hypothesis was also supported by the fact that replacement of the methyl group of xylamine by bromine in order to obtain DSP 4 did not change uptake inhibition of monoamines.

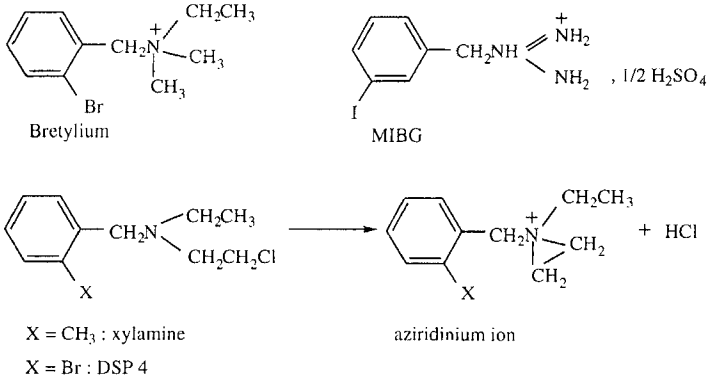


Figure 1

Moreover, the radioiodinated ligand (4c) could also be valuable for the visualization of the noradrenaline carrier by scintigraphic SPECT method. As for xylamine and DSP 4, we can expect that iodinated ligands (4b) and (4c) must link to the carrier via an aziridinium intermediate resulting from the ring closure of the 2-chloroethyl chain. In this case the ligand could be irreversibly bound to

the carrier without releasing this marker into storage vesicles. In order to explore these vesicles it would be of great value to use an iodinated analog of xylamine which could reversibly link to the noradrenaline transporter. We therefore synthesized the iodinated (2b) and radioiodinated (2c) ligands in which the 2-chloroethyl group was replaced by an ethyl group to avoid the formation of the aziridinium ion.

CONCLUSION

We synthesized two new iodinated analogs of xylamine N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b) and N,N-diethyl-2-iodobenzylamine (2b). These derivatives, as mIBG, show in vitro a low affinity for the noradrenaline uptake site. In view of the similar structures of these two compounds to that of mIBG (fig 1), we consider that they can be taken up and accumulated. Using original radiolabelling methods, we have obtained radioiodinated derivatives that could be used in vivo in animals. In vivo explorations in animals are now in progress.

EXPERIMENTAL PART

2-Iodobenzyl chloride, 2-bromobenzyl bromide, 2-(ethylamino)ethanol, diethylamine, thionyl chloride, glycine ethyl ester, ethyl bromide, lithium aluminium hydride (1M in ether) were purchased from Aldrich Chemical Company. [^{125}I]Sodium iodide was purchased from Amersham [37 MBq in 10 µL NaOH 0.1N, no-carrier-added, specific activity 74-81.4 TBq/mmol]. All solvents were usually redistilled and dried.Thin layer chromatographic analyses were conducted using silicagel 60F254 TLC plates from Merck and compounds were revealed by U.V. detection. Flash chromatography purification was conducted on silicagel 230-400 Mesh ASTM from Merck. ^{1}H-NMR spectra were recorded at 200 MHz on an AM 200 Brüker NMR spectrometer using tetramethylsilane as standard. I.R. spectra were recorded on a 1310 Perkin-Elmer spectrometer. Mass spectra were recorded on a MS-Engine Hewlett Packard 5989A. HPLC was performed using a Beckman 331 liquid chromatograph with a 254 nm U.V. detector. Radioactivity was determined with a Berthold LB 506 detector. The column utilized was a Chrompack 10 RP18, 25 cm x 4.6 mm, and the mobile phase was 0.05M phosphate (pH 2.55)/methanol (70/30,v/v) with a flow rate of 1 mL/min.

1- SYNTHESES

Synthesis of N,N-diethyl-2-bromobenzylamine (2a) and N,N-diethyl-2-iodobenzylamine (2b):

2-Bromobenzylbromide (1a) (2.50 g, 10 mmol) or 2-iodobenzylchloride (1b) (2.52 g, 10 mmol) and diethylamine (1.61 g, 22 mmol) were stirred in benzene (10 mL) for 12 h at room temperature, and heated at reflux for 2h. After cooling, the organic layer was extracted with 1N HCl (25 mL, then 2 x 10 mL). The aqueous phases were combined and washed with diethylether (3 x 10 mL). The acid phase was made alkaline with K_2CO_3. The benzylamine was extracted with diethylether (3 x 15 mL) and washed with brine (3 x 10 mL). The ether phase was dried with Na_2SO_4. The solvent was removed under reduced pressure and the benzylamine derivative (2a) (2.40 g, 9.9 mmol, 99 % yield) or the benzylamine derivative (2b) (2.75 g, 9.5 mmol, 95 % yield) was obtained as a colourless oil. The hydrochloride salt was precipitated in a 2N HCl ether solution.

Compound 2a:

Mass spectrum (EI, 70 eV): m/z 243 (10.6%), 241 (10%); 228 (78%), 226 (71.4%); 171 (97.4%), 169 (100%); 90 (23%); 86 (17.6%)

^{1}H-NMR ($CDCl_3$): δ = 1.07 [t, 6 H, ^{3}J = 7.1 Hz, $N(CH_2\underline{CH_3})_2$]; 2.58 [q, 4H, ^{3}J = 7.1 Hz, $N(\underline{CH_2}CH_3)_2$]; 3.66 (s, 2H, $Ar\underline{CH_2}N$); 7.08 (td, 1H arom, ^{3}J = 7.4 Hz, ^{4}J = 1.2 Hz); 7.29 (td, 1 H arom, ^{3}J = 7.4 Hz, ^{4}J = 0.8 Hz); 7.52 (dd, 1H arom, ^{3}J = 7.4 Hz, ^{4}J = 1.2 Hz); 7.58 (dd, 1H arom, ^{3}J = 7.4 Hz, ^{4}J = 0.8 Hz)

Compound 2b:

Mass spectrum (EI, 70 eV): m/z 289 (9%); 274 (82%); 217 (100%); 90 (20%); 86 (11%)

^{1}H-NMR ($CDCl_3$): δ = 1.07 [t, 6 H, ^{3}J = 7.1 Hz, $N(CH_2\underline{CH_3})_2$]; 2.59 [q, 4H, ^{3}J = 7.1 Hz, $N(\underline{CH_2}CH_3)_2$]; 3.58 (s, 2H, $Ar\underline{CH_2}N$); 6.93 (td, 1H arom, ^{3}J = 7.4 Hz, ^{4}J = 1.2 Hz); 7.32 (t, 1 H arom, ^{3}J = 7.4 Hz); 7.53 (d, 1H arom, ^{3}J = 7.4 Hz); 7.81 (dd, 1H arom, ^{3}J = 7.4 Hz, ^{4}J = 1.2 Hz)

Synthesis of N-(2-hydroxyethyl)-N-ethyl-2-bromobenzylamine (3a) and N-(2-hydroxyethyl)-N-ethyl-2-iodobenzylamine (3b):

2-Bromobenzyl bromide (1a) (2.50 g, 10 mmol) or 2-iodobenzyl chloride (1b) (2.52 g, 10 mmol) and 2-(ethylamino)ethanol (1.96 g, 22 mmol) were stirred in benzene (10 mL) for 12 h at room temperature, then refluxed for 2 h. After cooling, the benzylamino alcohol (3a or 3b) was extracted

with 1N HCl (25 mL, then 2 x 10 mL) ; the aqueous phases were combined and washed with diethylether (3 x 10 mL). The acid phase was made alkaline with K_2CO_3 and extracted with diethylether (3 x 15 mL). The organic phases were combined, washed with brine (3 x 10 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The benzylamino alcohol (2a) (2.25 g, 8.7 mmol, 87% yield) or the benzylamino alcohol (2b) (2.72 g, 8.9 mmol, 89% yield) was produced as a colourless oil.

Compound 3a:

Mass spectrum (EI, 70 eV): m/z 259 (1.4%), 257 (1.4%); 228 (78%), 226 (80%); 171 (99%), 169 (100%); 146 (2.29%); 102 (3.3%); 90 (20%)

^{1}H-NMR ($CDCl_3$): δ = 1.05 (t, 3 H, ^{3}J = 7.1 Hz, $NCH_2\underline{CH_3}$); 2.58 (q, 2H, ^{3}J = 7.1 Hz, $N\underline{CH_2}CH_3$); 2.67 (t, 2H, ^{3}J = 5.3 Hz, $N\underline{CH_2}CH_2OH$); 2.82 (s, 1H, $O\underline{H}$); 3.54 (t, 2H, ^{3}J = 5.3 Hz, $NCH_2\underline{CH_2}OH$); 3.70 (s, 2H, $Ar\underline{CH_2}N$); 7.10 (td, 1H arom, ^{3}J = 7.6 Hz, ^{4}J = 1.9 Hz); 7.26 (td, 1 H arom, ^{3}J = 7.6 Hz, ^{4}J = 1.2 Hz); 7.38 (dd, 1H arom, ^{3}J = 7.4 Hz, ^{4}J = 1.9 Hz); 7.52 (dd, 1H arom, ^{3}J = 7.4 Hz, ^{4}J = 1.2 Hz)

Compound 3b:

Mass spectrum (EI, 70 eV): m/z 305 (1.65%); 274 (94%); 217 (100%); 146 (1.98%); 102 (2.7%); 90 (18.3%)

^{1}H-NMR ($CDCl_3$): δ = 1.07 (t, 3 H, ^{3}J = 7.1 Hz, $NCH_2\underline{CH_3}$); 2.61 (q, 2H, ^{3}J = 7.1 Hz, $N\underline{CH_2}CH_3$); 2.68 (t, 2H, ^{3}J = 5.3 Hz, $N\underline{CH_2}CH_2OH$); 3.53 (t, 2H, ^{3}J = 5.3 Hz, $NCH_2\underline{CH_2}OH$); 3.65 (s, 2H, $Ar\underline{CH_2}N$); 6.94 (td, 1H arom, ^{3}J = 7.2 Hz, ^{4}J = 2.4 Hz); 7.33 (m, 2H arom); 7.83 (dd, 1H arom, ^{3}J = 7.8 Hz)

Synthesis of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (4a) and N-(2-chloroethyl)-N-ethyl-2-iodobenzylamine (4b):

N-(2-hydroxyethyl)-N-ethyl-2-bromobenzylamine (3a) (1.29 g, 5 mmol) or N-(2-hydroxyethyl)-N-ethyl-2-iodobenzylamine (3b) (1.52 g, 5 mmol) was dissolved in dried chloroform (5 mL). Thionyl chloride (0.89 g, 7.5 mmol) was added, the mixture was stirred for 2 h at room temperature and refluxed for 1 h. The solvent was evaporated under reduced pressure and cold water (20 mL) was

added. The aqueous phase was washed with diethylether (3 x 10 mL) and made alkaline with K_2CO_3 in the presence of diethylether (10 mL). The solution was extracted with diethylether (3 x 15 mL), combined phases were washed with brine (3 x 10 mL) after drying over Na_2SO_4. After filtration, 2N HCl in ether solution was slowly added to precipitate the hydrochloride salt. Two recrystallisations produced white crystals with 71% yield (1.11 g, 3.55 mmol) for derivative (4a) and 70% yield (1.26 g, 3.5 mmol) for derivative (4b).

Compound 4a:

Mass spectrum (EI, 70 eV): m/z 277 (3.8%), 275 (2.88%); 262 (4.6%), 260 (3.55%); 228 (88.8%), 226 (90.8%); 171 (96.8%), 169 (100%); 146 (1.21%); 120 (4.46%); 90 (19.5%)

^{1}H-NMR ($CDCl_3$): δ = 1.48 (t, 3 H, ^3J = 7.2 Hz, $NCH_2\underline{CH_3}$); 3.27 (q, 2H, ^3J = 7.2 Hz, $N\underline{CH_2}CH_3$); 3.40 (t, 2H, ^3J = 6.5 Hz, $N\underline{CH_2}CH_2Cl$); 4.00 (q, 2H, ^3J = 6.5 Hz, $NCH_2\underline{CH_2}Cl$); 4.50 (s, 2H, $Ar\underline{CH_2}N$); 7.30 (td, 1H arom, ^3J = 7.7 Hz, ^4J = 1.5 Hz); 7.47 (td, 1 H arom, ^3J = 7.6 Hz, ^4J = 1.2 Hz); 7.60 (dd, 1H arom, ^3J = 8 Hz, ^4J = 1.2 Hz); 8.34 (dd, 1H arom, ^3J = 7.8 Hz, ^4J = 1.5 Hz); 13.08 (broad s, 1 H, NH^+)

Compound 4b:

Mass spectrum (EI, 70 eV): m/z 323 (0.32%); 308 (0.32%); 274 (98.98%); 217 (100%); 146 (1.40%); 120 (4.82%); 90 (21.7%)

^{1}H-NMR ($CDCl_3$): δ = 1.49 (t, 3 H, ^3J = 7.1 Hz, $NCH_2\underline{CH_3}$); 3.28 (m, 2H, $N\underline{CH_2}CH_3$); 3.40 (t, 2H, ^3J = 5.2 Hz, $N\underline{CH_2}CH_2Cl$); 4.01 (m, 2H, $NCH_2\underline{CH_2}Cl$); 4.50 (s, 2H, ^3J = 5.6 Hz, $Ar\underline{CH_2}N$); 7.12 (td, 1H arom, ^3J = 7.8 Hz, ^4J = 1.2 Hz); 7.51 (td, 1 H arom, ^3J = 7.8 Hz, ^4J = 1 Hz); 7.90 (dd, 1H arom, ^3J = 7.8 Hz, ^4J = 1 Hz); 8.35 (dd, 1H arom, ^3J = 7.8 Hz, ^4J = 1.2 Hz); 12.90 (broad s, 1 H, NH^+)

Synthesis of N-(2-bromobenzyl)glycine ethyl ester (5a) and N-(2-iodobenzyl) glycine ethyl ester (5b):

2-Bromobenzylbromide (1a) (2.50 g, 10 mmol) or 2-iodobenzylchloride (1b) (2.52 g, 10 mmol) and glycine ethyl ester (2.27 g, 22 mmol) were stirred in ethanol (10 mL) for 12 h at room temperature

and then refluxed for 2 h. The solvent was removed under reduced pressure and 1N HCl (25 mL, then 2 x 10 mL) was added. The compound was extracted using the same procedure described above for the preparation of (3a) and (3b). A yellow oil was obtained with 55% yield (1.49 g, 5.5 mmol) for compound (5a) and with 65% yield (2.07 g, 6.5 mmol) for compound (5b).

Compound 5a:

Mass spectrum (EI, 70 eV): m/z 273 (3.06%), 271 (3.06%); 200 (87.56%), 198 (91.18%); 186 (32.22%), 184 (33.38%); 171 (100%), 169 (99.77%); 118 (13.17%); 90 (28,55%)

^{1}H-NMR ($CDCl_3$): δ = 1.25 (t, 3 H, ^{3}J = 7.2 Hz, $OCH_2\underline{CH_3}$); 2.05 (broad s, 1 H, NH); 3.40 (s, 2H, $Ar\underline{CH_2}N$); 3.88 (s, 2 H, $N\underline{CH_2}CO$); 4.16 (q, 2H, ^{3}J = 7.2 Hz, $O\underline{CH_2}CH_3$); 7.09 (td, 1H arom, ^{3}J = 7.5 Hz, ^{4}J = 2 Hz); 7.26 (td, 1 H arom, ^{3}J = 7.5 Hz, ^{4}J = 1.4 Hz); 7.38 (dd, 1H arom, ^{3}J = 7.5 Hz, ^{4}J = 2 Hz); 7.52 (dd, 1H arom, ^{3}J = 7.5 Hz, ^{4}J = 1.4 Hz)

Compound 5b:

Mass spectrum (EI, 70 eV): m/z 319 (6.25%); 246 (98.88%); 232 (42.56%); 217 (100%); 118 (12.52%); 90 (24.42%)

^{1}H-NMR ($CDCl_3$): δ = 1.25 (t, 3 H, ^{3}J = 7.1 Hz, $OCH_2\underline{CH_3}$); 2.07 (broad s, 1 H, NH); 3.40 (s, 2H, $Ar\underline{CH_2}N$); 3.82 (s, 2 H, $N\underline{CH_2}CO$); 4.16 (q, 2H, ^{3}J = 7.1 Hz, $O\underline{CH_2}CH_3$); 6.92 (td, 1H arom, ^{3}J = 7.6 Hz, ^{4}J = 2 Hz); 7.29 (td, 1 H arom, ^{3}J = 7.6 Hz, ^{4}J = 1.1 Hz); 7.36 (dd, 1H arom, ^{3}J = 7.6 Hz, ^{4}J = 2 Hz); 7.79 (dd, 1H arom, ^{3}J = 7.6 Hz, ^{4}J = 1.1 Hz)

Synthesis of N-ethyl-N-(2-bromobenzyl)glycine ethyl ester (6a) and N-ethyl-N-(2-iodobenzyl)glycine ethyl ester (6b):

Bromoethane (545 mg, 5 mmol) and K_2CO_3 (760 mg, 5.5 mmol) were added to N-(2-bromobenzyl)glycine ethyl ester (5a) (1.36 g, 5 mmol) or N-(2-iodobenzyl)glycine ethyl ester (5b) (1.59 g, 5 mmol) in ethanol (10 mL). The mixture was stirred for 12 h at room temperature and refluxed for 2 h. The solvent was removed under reduced pressure and cold water (20 mL) was added. The aqueous phase was extracted with diethylether (3 x 15 mL). The combined ether extracts were washed with brine (3 x 10 mL), dried over Na_2SO_4 and evaporated to give 70% yield (1.05 g,

3.5 mmol) for compound (6a) and 72% yield (1.25 g, 3.6 mmol) for compound (6b). The product was purified by flash chromatography on silica gel with ethylacetate/petroleum benzine (70/30, v/v) as mobile phase.

Compound 6a:

Mass spectrum (EI, 70 eV): m/z 301 (3.45%), 299 (3.40%); 272 (0.94%), 270 (0.90%); 228 (97.11%), 226 (100%); 171 (89.16%), 169 (87%); 146 (4.58%); 90 (21.98%)

^{1}H-NMR ($CDCl_3$): δ = 1.09 (t, 3H, ^3J = 7.1 Hz, $NCH_2\underline{CH_3}$); 1.27 (t, 3 H, ^3J = 7.1 Hz, $OCH_2\underline{CH_3}$); 2.75 (q, 2H, ^3J = 7.1 Hz, $N\underline{CH_2}CH_3$); 3.37 (s, 2H, $Ar\underline{CH_2}N$); 3.87 (s, 2 H, $N\underline{CH_2}CO$); 4.16 (q, 2H, ^3J = 7.1 Hz, $O\underline{CH_2}CH_3$); 7.09 (td, 1H arom, ^3J = 7.6 Hz, ^4J = 1.8 Hz); 7.28 (td, 1 H arom, ^3J = 7.6 Hz, ^4J = 1.2 Hz); 7.51 (dd, 1H arom, ^3J = 7.6 Hz, ^4J = 1.2 Hz); 7.57 (dd, 1H arom, ^3J = 7.6 Hz, ^4J = 1.8 Hz)

Compound 6b :

Mass spectrum (EI, 70 eV): m/z 347 (4.16%); 318 (1.31%); 274 (100%); 260 (1.71%); 217 (77.35%); 146 (3.49%); 90 (17.50%)

^{1}H-NMR ($CDCl_3$): δ = 1.08 (t, 3H, ^3J = 7.2 Hz, $NCH_2\underline{CH_3}$); 1.27 (t, 3 H, ^3J = 7.1 Hz, $OCH_2\underline{CH_3}$); 2.75 (q, 2H, ^3J = 7.2 Hz, $N\underline{CH_2}CH_3$); 3.37 (s, 2H, $Ar\underline{CH_2}N$); 3.80 (s, 2 H, $N\underline{CH_2}CO$); 4.16 (q, 2H, ^3J = 7.1 Hz, $O\underline{CH_2}CH_3$); 6.93 (td, 1H arom, ^3J = 7.6 Hz, ^4J = 1.7 Hz); 7.31 (td, 1 H arom, ^3J = 7.6 Hz, ^4J = 1.2 Hz); 7.52 (dd, 1H arom, ^3J = 7.6 Hz, ^4J = 1.7 Hz); 7.80 (dd, 1H arom, ^3J = 7.6 Hz, ^4J = 1.2 Hz)

2- RADIOLABELLING

Preparation of solution A and solution B:

Solution A: $SnSO_4$ (1 mg), gentisic acid (25 mg) and citric acid (35 mg) were dissolved in water (2.250 mL) and acetic acid (25 µL)

Solution B: $CuSO_4$, 5 H_2O (32.5 mg) was dissolved in water (10 mL)

Synthesis of N,N-diethyl-2-[^{125}I]iodobenzylamine (2c):

N,N-Diethyl-2-bromobenzylamine (2a) (1 mg, 3.9 µmol), acetic acid (45 µL), solution A (455 µL), solution B (30 µL) and [^{125}I] sodium iodide in 0.1N NaOH (5 µL, 18.5 MBq) were introduced into a sealed vial. The reaction mixture was stirred for 5 min and heated at 140°C in a sand bath for 45 min. After cooling, the pH was raised by adding solid K_2CO_3. The radiolabelled compound (2c) was extracted with diethylether (3 x 1 mL) and purified by HPLC (R_t = 9 min). The appropriate fraction was collected and the mobile phase was removed on a SEP-PAK C18 cartridge (Waters), using an excess of water. The radiolabelled compound was eluted with ethanol (500 µL). Ethanol was evaporated under nitrogen stream and the dried no-carrier-added radiolabelled compound (2c) was obtained with 30% yield. Compound (2c) was checked by coinjection with the compound (2b).

Synthesis of N-(2-chloroethyl)-N-ethyl-2-[^{125}I]iodobenzylamine (4c):

In a first step, N-ethyl-N-(2-bromobenzyl)glycine ethyl ester (6a) (1.17 mg, 3.9 µmol) was introduced in a sealed vial. The radiolabelled compound (6c) was produced using the same procedure described above for the preparation of (2c), and was obtained with 50% radiochemical yield. The compound (6c) was checked by coinjection with compound (6b) (R_t = 25 min). In a second step, anhydrous diethylether (300 µL) was added to the vial containing compound (6c). The reactor was cooled in an ice bath and then $LiAlH_4$ (1M in ether) (20 µL) was added. After 5 min, 10^{-1}M HCl (1 mL) was added for hydrolysation. The pH was raised by adding 1N NaOH and the solution was extracted with diethylether (2 x 1 mL) and dried over Na_2SO_4. After filtration diethylether was removed to dryness under a nitrogen stream. The radiolabelled compound (3c) was obtained with 80% radiochemical yield. The compound (3c) was checked by coinjection with compound (3b) (R_t = 8 min).

In a third step, anhydrous chloroform (250 µL) and thionyl chloride (25 µL) was added. The reaction mixture was heated at 40°C for 40 min. Chloroform was evaporated under a nitrogen stream. The radiolabelled compound (4c) was purified by HPLC (R_t = 15 min). The appropriate fraction was collected and the mobile phase was removed on a SEP-PAK C18 cartridge, using an excess of water. The radiolabelled compound was eluted with ethanol (500 µL). Ethanol was evaporated under nitrogen stream and the dried no-carrier-added radiolabelled compound (4c) was obtained with 30% yield. The compound (4c) was checked by coinjection with compound (4b) (R_t = 15 min).

3- BINDING EXPERIMENTS

In vitro binding experiments were performed according to Tejani-Butt (13) with minor modifications. Striatal membranes were prepared from the brains of Sprague Dawley rats. Tissues were homogenized in 10 vol 0.32 M sucrose using an Ultraturrax (Ultra-turrax T25). After 1000 g centrifugation for 10 min at 2°C, the supernatant was collected and the pellet homogenized and centrifuged as above. Supernatants were pooled and centrifuged at 17 500 g for 30 min at 2°C. Final pellets were suspended in a small volume of buffer and protein concentration was determined according to Bradford (14) using bovine serum albumin as standard. Binding assays were run in duplicate in a final volume of 500 µL containing of 400 µL of membrane proteins, 50 µL of [^{3}H]nisoxetine (NEN, Specific Activity 3026.6 GBq/mmol) and 50 µL of Tris/HCl buffer. For saturation assays [^{3}H]nisoxetine was used at a concentration of 0.3 nM to 10 nM, and for competition assays [^{3}H]nisoxetine was used at a concentration of 1 nM. Samples were incubated at 4°C for 4 h and filtered under reduced pressure on glass fiber filters GF/C (Whatman). Filters were washed three times with 4 mL ice-cold buffer; the radioactivity remaining on them was measured after addition of a scintillator (Optiphase Highsafe II, LKB) using a beta counter (LKB Rack Beta 1215). The specific binding was calculated by subtracting the non-specific binding defined in the presence of 10^{-5}M mazindol (RBI Bioblock) from the total binding.

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