ELSEVIER

European Journal of Pharmacology 391 (2000) 263–267

ejp

www.elsevier.nl/locate/ejphar

Desipramine binding to noradrenaline reuptake sites in cardiac sympathetic neurons in man in vivo

Andrea L. Malizia [a,*], Jan K. Melichar [a,b], Christopher G. Rhodes [b], Abel Haida [b], Alan H. Reynolds [c], Terry Jones [b], David J. Nutt [a]

[a] *Psychopharmacology Unit, University of Bristol, Bristol BS8 1TD UK*
[b] *MRC Cyclotron Unit, Hammersmith Hospital, London W12 0NN UK*
[c] *Wyeth Laboratories, Huntercombe Lane South, Taplow, Maidenhead, Berks, SL6 0PH UK*

Received 24 January 2000; accepted 28 January 2000

Abstract

Noradrenergic reuptake blockade is a recognised mechanism of antidepressant action, but the extent of the blockade necessary for therapeutic effect is not known and plasma levels do not provide a guide to therapy. We report a method to assess noradrenaline reuptake blockade in vivo in man using [^{11}C]*meta*-hydroxyephedrine and the multiple organs' coincidences counter. Eight healthy volunteers had two scans, one with tracer alone and one after preloading with desipramine 50–75 mg p.o. In all subjects, there was an increased washout rate of the radioligand from the heart following preloading ($t = 4.38$; $P < 0.003$) as well as a decrease of the area under the [^{11}C]*meta*-hydroxyephedrine time activity curve ($t = 7.4$; $P = 0.001$). In one subject who had three doses of desipramine, the increase in washout rate was dose-dependent. In conclusion, [^{11}C]*meta*-hydroxyephedrine in the multiple organs' coincidences counter gives a valid, low radiation method to assess noradrenergic reuptake blockade in the clinic.

Keywords: Noradrenaline reuptake site; *Meta*-hydroxyephedrine; PET (positron emission tomography); Tricyclic antidepressant; (Human)

1. Introduction

Drugs that block noradrenaline reuptake in presynaptic brain neurons have antidepressant properties. The exact mechanism by which they effect therapeutic change is unknown as there is a time-lag of at least 2 weeks between onset of blockade at the uptake site and clinical efficacy. Further, the effects of magnitude of blockade at the noradrenergic transporter on clinical efficacy have not been evaluated. In this study, we wish to investigate whether occupancy at noradrenaline reuptake sites could be assessed in man in vivo.

Desipramine is a secondary amine tricyclic with predominant noradrenaline reuptake blockade. The relation between the plasma kinetics of desipramine and clinical response has been extensively studied, but no firm conclusion about optimal plasma levels and desipramine efficacy has been reached, with most authors showing no relationship (e.g., Amsterdam et al., 1985). The pharmacokinetics of desipramine are nonlinear (Nelson and Jatlow, 1987) and related to cytochrome P450-2D6 activity (Spina et al., 1997), making single subject pharmacokinetic predictions difficult.

We sought to demonstrate that an index of desipramine occupancy at noradrenaline reuptake sites on sympathetic neurons in the heart can be obtained in vivo in man using [^{11}C]*meta*-hydroxyephedrine and the multiple organs' coincidences counter. [^{11}C]*meta*-hydroxyephedrine does not cross the blood-brain barrier, but can be used as a robust index of noradrenaline reuptake site occupancy in the heart. A number of potential noradrenaline reuptake site radioligands have been synthesised (e.g., Van Dort et al., 1997), but there are none to date that have desirable characteristics for brain quantification in that either they do not penetrate the blood-brain barrier or they have very high nonspecific binding that drowns the signal from specific binding. Thus, we selected cardiac sympathetic neurons as surrogate markers for the measurement of the occupation of noradrenaline reuptake sites in brain neurons.

* Corresponding author.: Tel.: +44-117-9253066; fax +44-117-9277057.
E-mail address: andrea.l.malizia@bristol.ac.uk (A.L. Malizia).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00103-5

^{11}C-meta-hydroxyephedrine

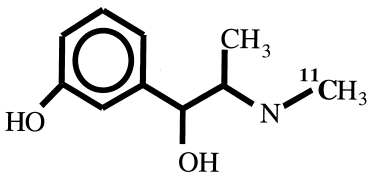

Fig. 1. Structure of meta-hydroxyephedrine.

[^{11}C]*meta*-hydroxyephedrine (Fig. 1) is a catecholamine analogue that enters presynaptic neurons by the noradrenaline reuptake site (Rosenspire et al., 1990). It has high affinity for the transporter so that upon release from the neuron it is taken up again by the uptake mechanism. This results in a minimal washout from the synaptic cleft. It is, therefore, an excellent marker of noradrenaline reuptake site kinetics and of presynaptic sympathetic integrity in the heart (Schwaiger et al., 1990; Melon et al., 1997). In the isolated rat heart tissue, half life for [^{11}C]*meta*-hydroxyephedrine is about 60 min (DeGrado et al., 1993). [^{11}C]*meta*-hydroxyephedrine reuptake can be blocked by drugs, such as desipramine, which have a high affinity for the noradrenaline transporter (DeGrado et al., 1993; Law et al., 1997).

The multiple organs' coincidences counter is a whole body γ-ray counter that has been modified to detect coincident counts from chosen regions and organs of the body (Fig. 2). The technique is very sensitive and can detect the low levels of emissions from radiolabelled tracers given at less than 1/100th the dose used in Positron Emission Tomography (PET). Reproducible time activity curves of exactly the same temporal profile to global time activity curves from PET can be obtained from the administration of 3–4 MBq of radiation using [^{11}C]-labelled ligands (such as flumazenil, diprenorphine and *m*-hydroxyephedrine) as opposed to 300–400 MBq with PET scanning protocols (Malizia et al., 1995), thus reducing the radiation exposure from 2–3 mSv to 20–30 μSv. Although there is loss of tomographic information, the multiple organs' coincidences counter has several advantages over conventional PET. Most importantly, the low dose of radioactivity (resulting in exposure levels equivalent to 3 days background) means that individuals can be investigated repeatedly (theoretically up to 200 times in 1 year and still keep within currently accepted limits for normal volunteers) so enabling longitudinal studies to be performed.

2. Materials and methods

2.1. Subjects

We recruited eight healthy male volunteers who had no significant medical or psychiatric history and who were on no current medication. All volunteers gave written informed consent. The research protocol was approved by the local Research Ethics Committee and by the Administration of Radioactive Substances Advisory Committee for the United Kingdom (ARSAC).

2.2. Synthesis of ^{11}C-meta-hydroxyephedrine

[^{11}C]*meta*-hydroxyephedrine was synthesised by direct methylation of *meta*-raminol by [^{11}C]iodomethane with no carrier added (Rosenspire et al., 1990). The amount of radioactivity in a given dose for the study ranged between 2.7 and 4.1 MBq with a total dose of 0.1–1 nmol *meta*-hydroxyephedrine.

2.3. Scanning procedures

The volunteers were scanned on two separate occasions in the multiple organs' coincidences counter. On one occasion, they received only an injection of [^{11}C]*meta*-hydroxyephedrine at the beginning of the scan — the tracer alone study. For the preloading study, they had the injection after taking desipramine orally 3 h beforehand (50–75 mg doses). On each occasion, the subjects were positioned in the supine posture so that there were two pairs of detectors recording the head and left chest time-activity curves over a 60-min period following injection of [^{11}C]*meta*-hydroxyephedrine (see Fig. 2). Blood samples for desipramine levels were taken at the time of [^{11}C]*meta*-hydroxyephedrine injection. In three studies, a further blood sample was taken 30 min after the injection (at mid-scan time). Coincident counts from each volume were collected every 5 sec throughout the scan. One volunteer had four studies in total: three preloading studies, taking three different doses of desipramine beforehand (50, 75 and 100 mg) and one-tracer-alone study.

2.4. Analysis

Data were analysed using Matlab (The Mathworks). Counts were summed over 1-min intervals and normalised

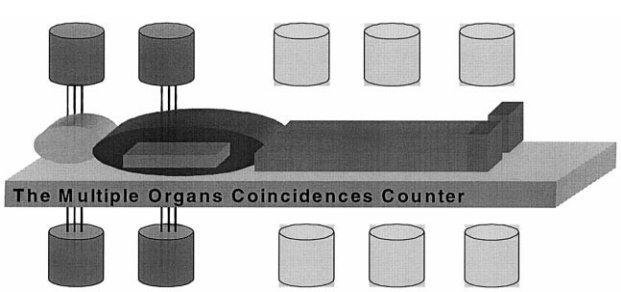

Fig. 2. Schematic representation of a whole body gamma counter modified to detect coincidences. Detectors are sodium iodide crystals placed above and below the supine subject.

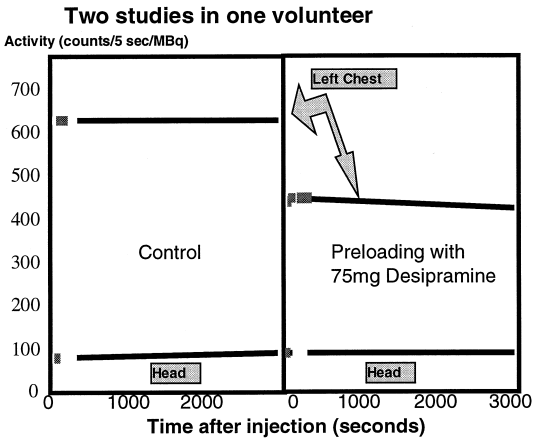

Fig. 3. Typical fitted time [^{11}C]meta-hydroxyephedrine activity curves in tracer alone and preloading experiments. Note the much smaller signal from the head as [^{11}C]meta-hydroxyephedrine does not cross the blood-brain barrier and, therefore, the signal is only from blood and adventitia. Note the increase in washout in the preloading experiment as well as the decrease in AUC.

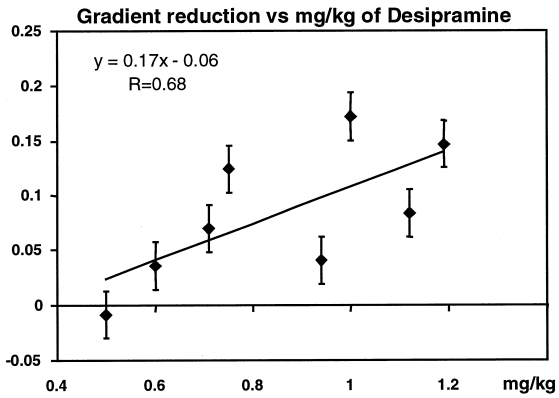

Fig. 4. Relationship between desipramine dose and [^{11}C]meta-hydroxyephedrine washout in different subjects.

to injected activity. The slope of the time activity curves was determined in all experiments using a linear least squares fit. The time segment selected for analysis was 5 to 55 min (Fig. 3). The area under the curve (AUC) was also determined for this period. The data collected in the first 5 min uptake phase were excluded. The clearance of [^{11}C]*meta*-hydroxyephedrine has been shown to have a $t_{1/2}$ of 60 min in the isolated perfused rat heart (DeGrado et al., 1993), indicating that its washout is slow and, thus, can be approximated by a linear fit.

2.5. Plasma levels

Blood samples were collected into tubes containing Lithium heparin and spun in a centrifuge. The plasma was separated and stored at −20°C until required for assay by high-performance liquid chromatography (HPLC).

3. Results

Eight male volunteers aged 24–36 (average 28 S.D. 4.3) weighing 63–100 kg (average 76 S.D. 12.1) took part in the study. Two of the blood levels (one from the multiple dose subject) were not available for analysis.

3.1. Chest time activity curves

There was an increased washout rate of [^{11}C]*meta*-hydroxyephedrine after preloading with desipramine (paired $t = 4.38$; $P < 0.003$), as well as a decrease in the AUC (paired $t = 7.4$; $P = 0.001$) in all paired control to preload studies (i.e., not including the two additional studies in one subject) (see Table 1). A typical paired study is shown (Fig. 3). The change in slope correlated significantly with the dose/kg administered ($r = 0.68$ one-tailed $P < 0.05$) (Fig. 4), but not with the plasma levels ($r = 0.50$). Neither

Table 1
Summary of findings using oral desipramine preloading in the multiple organs coincidences counter
This shows all the results used to calculate the various results presented in the paper (n/a: result is unavailable).

Desipramine doses			Blood results	Calculated gradients[a] (counts/min/MBq)			Calculated area under the curve (counts/MBq)		
Dose (mg)	Weight (kg)	DMI dose (mg/kg)	DMI plasma levels (ng/ml)	Control scan	Preload scan	Gradient reduction	Control scan	Preload scan	% Change in AUC (%)
75	63	1.19	20	0.093	−0.054	0.147	41874	30703	−27
75	67	1.12	(n/a)	0.109	0.026	0.083	43964	25074	−43
75	75	1.00	15.6	0.141	−0.031	0.172	25031	12229	−51
75	80	0.94	19.6	0.031	−0.009	0.041	30580	21549	−30
50	67	0.75	16.1	0.102	−0.022	0.124	32110	22036	−31
50	70	0.71	23.5	0.086	0.016	0.070	36182	28247	−22
50	84	0.60	12.27	0.031	−0.004	0.036	30580	20483	−33
50	100	0.50	3.6	−0.030	−0.022	−0.008	19317	15963	−17

[a] Parameters from left chest curves.

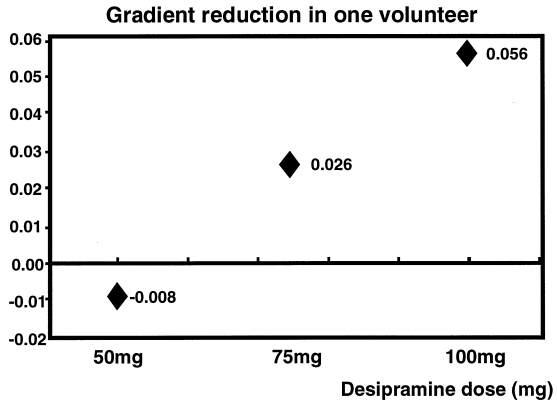

Fig. 5. Linear relationship in one subject between desipramine dose administered and [^{11}C]meta-hydroxyephedrine washout.

dose/kg administered ($r = 0.16$) nor plasma levels ($r = 0.56$) correlated significantly with the AUC changes.

The change in washout rate for the subject who had three doses of desipramine was dose-dependent (0.5 mg/kg: 0.008, 0.75 mg/kg: −0.026, 1 mg/kg: −0.056) (Fig. 5).

3.2. Head time activity curves

There were small nonsignificant changes in the slope and AUC of the head time activity curves (e.g., Fig. 3).

3.3. Plasma levels

There was no significant correlation between plasma levels and dose/kg given ($r = 0.59$). Three subjects had a second sample of desipramine taken at 30 min into the scan, which confirmed that there were no large changes in plasma desipramine concentration during the scan (16.1 and 15.8, 12.3 and 10.9, 23.5 and 30.1 ng/ml).

4. Discussion

This study demonstrates that it is possible to identify desipramine occupation at noradrenaline reuptake sites on cardiac nerve endings using [^{11}C]*meta*-hydroxyephedrine and global heart monitoring using the multiple organs' coincidences counter. This means that for the first time we have a tool that will allow indices of occupation to be related to clinical response to antidepressants that have significant noradrenaline reuptake blockade. Furthermore, this tool will be useful in early human drug development for proof of principle of noradrenergic reuptake site blockade, as well as helping with initial dose-ranging studies.

Both washout slope and AUC of the time activity curves were altered by desipramine preloading consistent with blockade of noradrenergic reuptake sites. The observation that significant correlation is only achieved between dose-administered and rate of washout is probably due the small sample; however, blood levels probably do not reflect synaptic concentration and there was no significant correlation between plasma levels and dose administered. Since, at best, the relationship accounts for less than 30% of the variance, it is likely that these results explain the hitherto observed lack of correlation between plasma levels and therapeutic efficacy (Spina et al., 1997).

In all subjects, we found that the AUC of the time activity curves was altered by noradrenergic reuptake blockade to a greater extent than the rate of clearance. This would not have been predicted from rat studies (DeGrado et al., 1994), where clearance was shown to increase up to threefold with no apparent reduction in tissue uptake. The implication is that the affinity of *meta*-hydroxyephedrine for the human noradrenaline transporter is lower than for the rat transporter so that delivery to the human tissue is not the limiting factor in signal magnitude as it is in rat. This notion is supported by the previously observed differences in heart volume of distribution between the two species, this being 70 in man and 230 in rat (DeGrado et al., 1994; Schafers et al., 1998).

The obvious limitation of this methodology is that it is not possible to separate tomographically the various types of tissue in the field of view and, therefore, the signal is composite. Furthermore, subnanomolar injection of [^{11}C]*meta*-hydroxyephedrine means that it is impossible to obtain accurate blood levels of radioligand. This precludes a measurement of the [^{11}C]*meta*-hydroxyephedrine input function and the use of kinetic analysis to provide uptake parameters independent of blood flow. Thus, the data are subject to additional noise from changes in delivery and extraction of the tracer and from recirculating labelled metabolites.

However, there are also considerable advantages; the very low amount of radioactivity used (< 1% of traditional PET scans) and the low cost of the equipment means that these studies can be performed many times in the same individuals, thus allowing the technique to be applied to sequential pharmacological studies in volunteers of either sex.

The apparent linear relationship between increases in administered dose and the increased [^{11}C]*meta*-hydroxyephedrine washout indicated that this method may allow titration of dose and occupancy leading to the possibility of studying dose–effect relationship in patients. Thus, we predict that this methodology will be most powerful in defining intra-individual changes with dose increases as, inter-subjects, differences in kinetics result in poorer signal-to-noise definition.

Acknowledgements

ALM was a Wellcome Training Fellow. We are grateful to John Wyeth and brother for financial support. Dr Mike Franklin, Oxford Psychopharmacology Unit, analysed the desipramine plasma samples.

References

Amsterdam, J.D., Brunswic, D.J., Potter, L., Winokur, A., Rickels, K., 1985. Desipramine and 2-hydroxydesipramine plasma levels in endogenous depressed patients. Lack of correlation with therapeutic response. Arch. Gen. Psychiatry 42, 361–364.

DeGrado, T.R., Hutchins, G.D., Toorongian, S.A., Wieland, D.M., Schwaiger, M., 1993. Myocardial kinetics of carbon-11-*meta*-hydroxyephedrine: retention mechanisms and effects of norepinephrine. J. Nucl. Med. 34, 1287–1293.

Law, M.P., Osman, S., Davenport, R.J., Cunningham, V.J., Pike, V.W., Camici, P.G., 1997. Biodistribution and metabolism of [*N*-methyl-^{11}C]-*m*-hydroxyephedrine in the rat. Nucl. Med. Biol. 24, 417–424.

Malizia, A.L., Forse, G., Gunn, R., Haida, A., Melichar, J., Poole, K., Bateman, D., Fahy, D., Nutt, D., Jones, T., 1995. A new human (psycho)pharmacology tool: the multiple organs coincidences counter. J. Psychopharmacol. 9, 294–306.

Melon, P.G., Boyd, C.J., McVey, S., Mangner, T.J., Wieland, D.M., Schwaiger, M., 1997. Effects of active chronic cocaine use on cardiac sympathetic neuronal function assessed by carbon-11-hydroxyephedrine. J. Nucl. Med. 38, 451–456.

Nelson, J.C., Jatlow, P.I., 1987. Nonlinear desipramine kinetics: prevalence and importance. Clin. Pharmacol. Ther. 41, 666–670.

Rosenspire, K.C, Haka, M.S., Van Dort, M.E., Jewett, D.M., Gildersleeve, D.L., Schwaiger, M., Wieland, D.M., 1990. Synthesis and preliminary evaluation of carbon-11-*meta*-hydroxyephedrine: a false transmitter agent for heart neuronal imaging. J. Nucl. Med. 31, 1328–1334.

Schafers, M., Dutka, D., Rhodes, C.G., Lammertsma, A.A., Hermansen, F., Schober, O., Camici, P.G., 1998. Myocardial presynaptic and postsynaptic autonomic dysfunction in hypertrophic cardiomyopathy. Circ. Res. 82, 57–62.

Schwaiger, M., Kalff, V., Rosenspire, K., Haka, M.S., Molina, E., Hutchins, G.D., Deeb, M., Wolfe, E. Jr., Wieland, D.M., 1990. Noninvasive evaluation of sympathetic nervous system in human heart by positron emission tomography. Circulation 82, 457–464.

Spina, E., Gitto, C., Avenoso, A., Campo, G.M., Caputi, A.P., Perucca, E., 1997. Relationship between plasma desipramine levels, CYP2D6 phenotype and clinical response to desipramine: a prospective study. Eur. J. Clin. Pharmacol. 51, 395–398.

Van Dort, M.E., Kim, J.H., Tluczek, L., Wieland, D.M., 1997. Synthesis of ^{11}C-labeled desipramine and its metabolite 2-hydroxydesipramine: potential radiotracers for PET studies of the norepinephrine transporter. Nucl. Med. Biol. 24, 707–711.