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Synthesis and Regional Mouse Brain Distribution of [11C]Nisoxetine, a Norepinephrine Uptake Inhibitor

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Nisoxetine, a selective and high affinity (IC_{50} = 1 nM) inhibitor of NE reuptake, has been radiolabeled in high specific activity (>600 Ci/mmol) by the alkylation of the nor-methyl precursor with [^{11}C]CH_3I. Synthetic yields are good (40–60% from [^{11}C]methyl iodide, corrected for decay, 20 min synthesis), with the product purified by HPLC. *In vivo* studies of regional brain distribution in CD-1 mice show uptake and retention of tracer in the cortex, striatum, hypothalamus and thalamus, with the highest levels in the hypothalamus and cortex. Specific binding in the cortex and hypothalamus can be reduced by preadministration of 7 mg/kg i.v. unlabeled nisoxetine. The possible value of [^{11}C]nisoxetine as a PET imaging agent is discussed.

Introduction

Nisoxetine [DL-*N*-methyl-3-(*o*-methoxyphenoxy)-3-phenylpropylamine; Fig. 1] is a high affinity (IC_{50} = 1 nM) inhibitor of the presynaptic norepinephrine (NE) reuptake system (Wong and Bymaster, 1976; Wong *et al.*, 1982). It has good selectivity for the NE system (serotonin uptake, IC_{50} = 1000; dopamine uptake, IC_{50} = 360) and has little affinity for neurotransmitter receptors. The characteristics make nisoxetine an excellent candidate for use in *in vivo* studies of the NE uptake system. As part of our program to prepare new radiopharmaceuticals for positron emission tomography (PET), based on the neurotransmitter uptake systems (Kilbourn *et al.*, 1989), we have prepared ($t_{1/2}$ = 20.4 min)^{11}C-labeled nisoxetine and have begun the evaluation of the time course, regional selectivity and specificity of this new radiotracer in rodent brain.

Materials and Methods

Chemicals

Synthesis of [^{11}C]nisoxetine. Carbon-11 was produced by proton irradiation of a nitrogen gas target. The radioactivity obtained in the cyclotron target (mainly $^{11}CO_2$) was converted to [^{11}C]methyl iodide by modifications of established procedures (Marazano *et al.*, 1977; Jewett, 1987). [^{11}C]Nisoxetine was prepared by the alkylation of the nor-methyl derivative (1 mg, free base) in 250 μL DMF/DMSO (3:1) for 5 min at 100°C. Purification and isolation was by HPLC using an alumina column (Maxsil 5 μm) and a gradient of ethanol in chloroform (10%, 0–7 min, linear gradient 10–20%; 7–9 min, hold at 20% for 4 min; flow rate 2 mL/min: R_t nisoxetine = 10–12 min; R_t nornisoxetine > 30 min). The organic solvents were removed by rotary evaporation and the product formulated in saline containing a trace amount of HCl. Decay-corrected yields of [^{11}C]nisoxetine (based on [^{11}C]methyl iodide) were 40–60%, with a radiochemical purity of >95% (TLC, silica gel, 95:5 chloroform/ethanol, R_f = 0.3) and sp. act. > 600 Ci/mmol (at EOS).

Animal studies

CD-1 mice (Charles River; 25–30 g) were anesthetized (ether) and injected with 10–300 μCi of [^{11}C]nisoxetine in saline–HCl solution via the tail vein. At designated times the animals were sacrificed by decapitation and the brain rapidly removed and dissected [modifications of the method of Glowinski and Iversen (1966)]. Tissue samples were weighed, then counted in an automatic γ-counter.

For blocking studies animals were injected via the tail vein with nisoxetine hydrochloride (7 mg/kg, saline solution), 20 min prior to radiotracer injection. Control animals were injected with vehicle alone. Data were analyzed using a two-tailed Students *t*-test: *P*-values are given in Table 1.

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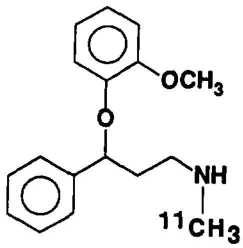

Fig. 1. Structure of [^{11}C]nisoxetine.

Results

Synthesis of [^{11}C]nisoxetine

The synthesis of [^{11}C]nisoxetine is very simple, involving the alkylation of nor-methyl nisoxetine with high specific activity, no-carrier-added [^{11}C]methyl iodide. Under these conditions, utilizing an excess of the primary amine and no added base, we observe clean mono-methylation at the no-carrier-added level. The product is separated from the precursors (nor-nisoxetine, [^{11}C]methyl iodide and [^{11}C]methanol) by an alumina HPLC column.

Time course and regional brain uptake

The time course of [^{11}C]nisoxetine distribution in mouse brain is shown in Table 1. Initial uptake is nearly uniform through all brain tissues, and with increasing time radioactivity levels in all brain tissues slowly decrease. Blood levels of carbon-11 are actually higher at 10 min than at 2 min, but then also slowly decrease with time. [^{11}C]Nisoxetine levels in brain are highest at 2 min and only decrease about 30% within the 60-min experiment. At 60 min the rank order of radioactivity in different brain regions is hypothalamus = cortex > cerebellum = striatum = thalamus. High initial uptake is seen in the heart followed by a rapid decrease, and considerable lung activity is seen at 60 min.

Blocking studies

Pretreatment with nisoxetine (7 mg/kg i.v.) caused a statistically significant decrease in radiotracer concentrations in the cortex and hypothalamus, with little effect in other areas (Table 1). Whole brain radioactivity (%ID/organ) was decreased by 20%.

Discussion

Nisoxetine (Fig. 1) is a simple chemical structure with remarkable selectivity for the NE reuptake system. Although it has been previously prepared in carbon-14 form (Wong and Bymaster, 1976), the low specific activity obtained with that radionuclide (3.48 μCi/mg) did not allow for *in vivo* study of this compound at true tracer levels. The use of carbon-11 allows the study of nisoxetine *in vivo* at a much higher specific activity (>600 Ci/mmol). We have successfully prepared this radiotracer by the simple N-alkylation of the nor-methyl derivative with high specific activity [^{11}C]methyl iodide, with the product isolated by HPLC.

The regional distribution of [^{11}C]nisoxetine in mouse brain is consistent with the rank order previously reported for rat brain using *in vitro* autoradiography and [^{3}H]desmethylimipramine (Lee *et al.*, 1982; Biegon and Rainbow, 1983) or [^{3}H]mazindol (Javitch *et al.*, 1985). Binding is higher in the cortex and hypothalamus than in the striatum, cerebellum or thalamus. The gross dissection technique utilized in this work does not allow finer determinations of regional radiotracer binding, and thus we cannot examine if the binding of [^{11}C]nisoxetine will show the regional variation apparent for noradrenaline concentrations in rodent brain (Versteeg *et al.*, 1976) or *in vitro* binding of [^{3}H]desmethylimipramine (Lee *et al.*, 1982; Biegon and Rainbow, 1983). Measurement of radioactivity in very small brain regions is difficult as methodological errors are introduced in the excision, weighing and counting of such small samples of tissue. We have previously utilized *in vivo* autoradiography with an ^{18}F-labeled dopamine uptake inhibitor, [^{18}F]GBR 13119 (Kilbourn *et al.*, 1988), and have obtained a more precise definition of the regional brain uptake patterns than was possible using dissection tech-

Table 1. *In vivo* distribution of [^{11}C]nisoxetine in mouse brain

	Time (min)				
	2	10	20	60	60+ nisoxetine
		% *ID/g*			
Striatum	1.46 ± 0.20	1.21 ± 0.41	0.64 ± 0.61	0.71 ± 0.13	0.76 ± 0.13
Cerebellum	1.49 ± 0.25	1.0 ± 0.36	1.01 ± 0.23	0.92 ± 0.12	0.76 ± 0.07
Cortex	1.38 ± 0.28	1.12 ± 0.43	1.18 ± 0.35	1.13 ± 0.099	0.92 ± 0.10*
Hypothalamus	1.4 ± 0.29	1.04 ± 0.28	1.34 ± 0.75	1.19 ± 0.31	0.59 ± 0.24**
Thalamus	1.41 ± 0.25	1.1 ± 0.15	0.79 ± 0.12	0.81 ± 0.29	0.64 ± 0.23
Blood	1.28 ± 0.17	1.72 ± 0.5	1.51 ± 0.20	0.84 ± 0.25	0.82 ± 0.23
Heart	12.14 ± 1.5	3.7 ± 0.69	3.4 ± 0.41	1.04 ± 0.25	1.23 ± 0.31
Lung	—	—	—	5.16 ± 2.9	7.2 ± 1.5
		% *ID/organ*			
Brain	0.71 ± 0.12	0.51 ± 0.15	0.53 ± 0.11	0.513 ± 0.08	0.403 ± 0.05***
		Ratios			
Cortex/striatum	0.96 ± 0.07	1.17 ± 0.27	1.72 ± 0.69	1.62 ± 0.41	1.21 ± 0.19****
Hypo/striatum	0.96 ± 0.16	1.41 ± 0.79	1.96 ± 1.4	1.51 ± 0.67	0.87 ± 0.09****

Data shown are time course in control animals, and distribution in mouse brain 60 min after prior administration of 7 mg/kg nisoxetine. Data are given as mean + SD.

*P = 0.009; **P = 0.04; ***P = 0.08; ****P = 0.06.

niques similar to those used here. Thus, more exact determinations of nisoxetine binding sites in brain will await autoradiographic studies using [^{11}C]nisoxetine or an ^{18}F-labeled derivative.

Only a portion of the radioactivity in the target tissues (cortex, hypothalamus) can be blocked by pretreatment with pharmacological doses of nisoxetine (7 mg/kg i.v.). The blocking study resulted in reductions of the cortex/striatum and hypothalamus/striatum ratios (Table 1) but not in the cortex/cerebellum ratio (data not shown). Decreases in the specific binding of [^{11}C]nisoxetine in the cortex and hypothalamus, but not the striatum, are consistent with decreases in *in vitro* NE uptake or binding of NE uptake inhibitors ([^{3}H]desipramine and [^{3}H]mazindol) by brain synaptosomes after *in vivo* treatment of rodents with 6-hydroxydopamine (Biegeon and Rainbow, 1983) or DSP-4 [*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (Jonsson *et al.*, 1981; Lee *et al.*, 1982; Logue *et al.*, 1985)]. The results obtained in this study suggest considerable (60–75%) levels of non-specific binding for [^{11}C]nisoxetine (with non-specific binding assumed to include binding to other receptor or uptake sites as well as low affinity high capacity sites). Such levels are similar to those found with other lipophilic amines, such as [^{11}C]cyanoimipramine [serotonin uptake inhibitor (Hashimoto *et al.*, 1987)] and [^{11}C]pyrilamine [histamine H-1 receptor antagonist (Yanai *et al.*, 1988)] when studied in similar protocols in rodent brain. In this study we have made the assumption that the *in vivo* binding characteristics of nisoxetine will be identical to those *in vitro* (low affinity for DA or 5-HT uptake systems; little affinity for any receptors); these assumptions need to be tested. Lipophilic labeled metabolites are unlikely, but have not been ruled out for [^{11}C]nisoxetine: the expected mode of metabolism should be via N-demethylation to form [^{11}C]methanol, and in preliminary experiments (data not shown) the radioactivity in blood remains as lipophilic material throughout the study.

The data reported here constitute the very first *in vivo* study of a high specific activity radiolabeled NE uptake inhibitor. Non-specific binding of [^{11}C]nisoxetine appears high, which may diminish its ultimate value as a PET imaging agent. Unfortunately, there are few other candidate compounds which might be radiolabeled. Tomoxetine [*N*-methyl-3-(*o*-methylphenoxy)-3-phenylpropylamine (Wong *et al.*, 1982)]; is structurally very similar and will likely suffer the same problems in non-specific binding. Modifications of either nisoxetine or tomoxetine will require extensive *in vitro* and *in vivo* analyses to determine the effects of structural changes on affinity and selectivity for the NE uptake system. Most studies of the NE uptake system (all *in vitro*) have utilized [^{3}H]desmethylimipramine, a compound which has been shown to bind to a second site *in vitro* and whose selectivity *in vivo* is unknown. Other candidates, such as nomifensine, show considerable cross-reactivity with the other monoamine reuptake systems (Fielding and Szewczak, 1984), which would complicate *in vivo* applications. For these reasons we are continuing to examine the properties of nisoxetine *in vivo*, and in particular its kinetic properties in primates, as well as the changes of specific nisoxetine binding after chemical lesions (e.g. DSP-4) or pharmacological treatments [MAO inhibitors and reserpine (Lee *et al.*, 1983)] which should alter NE uptake sites. Such studies may be of importance in understanding the *in vivo* behavior of NE uptake inhibitors, and have not been attempted to date due to the lack of a ligand of good selectivity and high specific activity.

Nisoxetine has also been reported as a high affinity inhibitor of NE uptake by synaptosomes prepared from heart tissue (Wong *et al.*, 1975). Although there is high initial heart uptake of [^{11}C]nisoxetine, washout of radioactivity is rapid. This reuptake inhibitor is thus different than 2-iodo-desmethylimipramine, previously proposed as a "chemical microsphere" due to high extraction and retention in heart tissue (Little *et al.*, 1986). The low heart/lung ratio obtained with [^{11}C]nisoxetine at 60 min does not support development of this radiotracer for the study of the noradrenergic innervation of the heart.

Summary

We have prepared [^{11}C]nisoxetine, a high affinity NE reuptake inhibitor, in high specific activity. *In vivo* studies in mice show adequate brain penetration and good retention of radioactivity, although the greater proportion of such appears to be by non-specific binding. Regional distribution of radioactivity is consistent with the known distribution of noradrenergic nerve terminals. Future evaluation of this radiotracer will include measurements of [^{11}C]nisoxetine in DSP-4-treated rats and mice, kinetic studies of the regional brain distribution in primates using PET and the determination of possible metabolites in blood and tissue.

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