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SELECTIVE, IRREVERSIBLE IN VIVO BINDING OF [^{11}C]CLORGYLINE AND [^{11}C]-L-DEPRENYL IN MICE: POTENTIAL FOR MEASUREMENT OF FUNCTIONAL MONOAMINE OXIDASE ACTIVITY IN BRAIN USING POSITRON EMISSION TOMOGRAPHY

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Monoamine oxidase (EC 1.4.3.4, amine:O_2 oxidoreductase, MAO), which catalyzes the oxidative deamination of a variety of monoamines, has been subdivided, on the basis of substrate and inhibitor selectivity, into two types: MAO-A and MAO-B. The A form selectively oxidizes 5-hydroxytryptamine and is selectively and irreversibly inhibited by clorgyline. The B form selectively oxidizes benzylamine and is inhibited by L-deprenyl (1). MAO has been the subject of a large number of studies directed toward determining whether either of these enzyme forms might be of significance to the etiology, diagnosis or treatment of medical and psychiatric disorders (2).

The advent of positron emission tomography (PET) has made possible the study of metabolism and physiological processes in health and disease in the human body utilizing organic molecules labeled with short-lived positron emitting nuclides (3). The MAO inhibitors (MAOIs) clorgyline and L-deprenyl act as suicide inhibitors, deactivating the enzyme by the formation of a covalent bond to its active site (4). By labeling these two MAOIs with a positron emitting nuclide it might be possible to label selectively each form of MAO in vivo and to determine quantitatively the patterns of distribution of the labeled MAO using PET.

As a continuation of a program to investigate the synthesis and use of positron emitter labeled radiotracers to probe functional MAO activity in vivo (5-7), we have synthesized clorgyline and L-deprenyl labeled at the N-methyl carbon with carbon-11, a positron emitter with a half-life of 20.4 min. The advantage of this approach, relative to another strategy which was described recently (8), is the potential of selectively assaying MAO A or B using tracers whose inhibitory profiles are well characterized and whose use has provided the basis for the development of an extensive data base on MAO A and B. The first phase of this study was to determine whether the in vivo rates and specificities of the ^{11}C-labeled MAOIs bonding MAO would allow their use in the study of the A and B forms of this enzyme using the PET methodology.

METHODS

[^{11}C]Clorgyline and [^{11}C]-L-deprenyl were synthesized by the alkylation of the corresponding desmethyl amines with [^{11}C]methyl iodide (manuscript in preparation). The specific activity of these tracers was ~ 200 mCi/μmole at the end of synthesis, and the synthesis time was 45 min.

Male Swiss albino mice (BNL strain) were injected intravenously with 100-200 μCi (~ 1 nmole) of [^{11}C]MAOI and killed by cervical fracture at 5, 30 and 60 min post injection. Organs were rapidly removed, blotted free of blood, placed in preweighed counting vials, and counted, along with injection standards, in a sodium iodide well counter.

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For the determination of protein binding, freshly excised brains were minced in preweighed centrifuge tubes containing 2 ml 0.4% $HClO_4$ (0.4 M), weighed, counted, diluted with 1 ml $HClO_4$ and 1 ml methanol, and homogenized by ultrasonication. After centrifugation and decantation, the pellets were resuspended in 3 ml methanol, sonicated, and recentrifuged. The combined supernatant fractions and pellet were counted in a sodium iodide well counter. The efficiency of this method in extracting free MAOIs was demonstrated by adding [^{11}C]MAOIs to the brains of untreated mice before homogenization. Recovery in the supernatant fraction was > 90%. Because of the short half-life of C-11, corrections for the decay of the isotope were calculated to a standard time (end of cyclotron bombardment) for all data.

For pretreatment experiments, mice were given intraperitoneal injections of unlabeled clorgyline or L-deprenyl (10 mg/kg) 1 h before the administration of the ^{11}C-tracer. For example, clorgyline was followed by [^{11}C]clorgyline (pretreatment) or by [^{11}C]-L-deprenyl (cross pretreatment).

RESULTS

The tissue distribution and clearance patterns of [^{11}C]clorgyline are summarized in Fig. 1a, while those of [^{11}C]-L-deprenyl are given in Fig. 1b. Also shown is the effect of pretreatment with the unlabeled compound. In two separate studies of cross inhibition ([^{11}C]clorgyline pretreated with L-deprenyl and [^{11}C]-L-deprenyl pretreated with clorgyline) the patterns of uptake and clearance of radioactivity were, for the most part, similar to those observed in the control study (data not shown).

The fractions of membrane bound radioactivity in brains in control, pretreated and cross pretreated mice are given in Fig. 2.

DISCUSSION

One of the aims of this investigation was to determine if the biodistribution of [^{11}C]MAOIs was specifically associated with the presence of metabolically active MAO subtypes or if nonspecific distribution and binding would effectively mask any specific interactions. As can be seen in Fig. 1, the clearance patterns of [^{11}C]clorgyline and [^{11}C]-L-deprenyl differ in some organs of control animals. The retention of L-deprenyl in heart contrasts with the clearance of clorgyline from this organ with a similar, although less dramatic, pattern being observed for lung. After pretreatment with L-deprenyl, these organs clear [^{11}C]-L-deprenyl as rapidly as [^{11}C]clorgyline, suggesting that MAO-B is the principal MAO subtype in mouse heart and lung in agreement with previous work (9). The results for liver, which is also predominantly type B, appear to be nonspecific.

In brain, [^{11}C]clorgyline and [^{11}C]-L-deprenyl display similar patterns to each other in both control and pretreated mice, with clorgyline showing a slight but consistently higher uptake than L-deprenyl, indicating the comparable presence of MAO A and B in mouse brain. As can be seen from Fig. 2, the effect of pretreatment was a specific one in that cross pretreatment, e.g. clorgyline administration prior to [^{11}C]-L-deprenyl and L-deprenyl pretreatment prior to [^{11}C]clorgyline, did not alter significantly from control the patterns of [^{11}C]-L-deprenyl or [^{11}C]clorgyline disposition respectively. Furthermore, the specificity of the inhibition of membrane binding in brain of [^{11}C]MAOIs by pretreatment relative to control animals is even more dramatic (see Fig. 2), indicating that covalent binding (and probably suicide inhibition) of MAO is indeed responsible for the pattern of radioactivity distribution observed in brain. In addition, the ^{11}C binding to membrane is essentially complete by 30 min in mice and is therefore compatible with the short half-life of carbon-11. In support of this, preliminary results of high pressure liquid radiochromatographic analysis of brain supernatant fractions indicate that less than

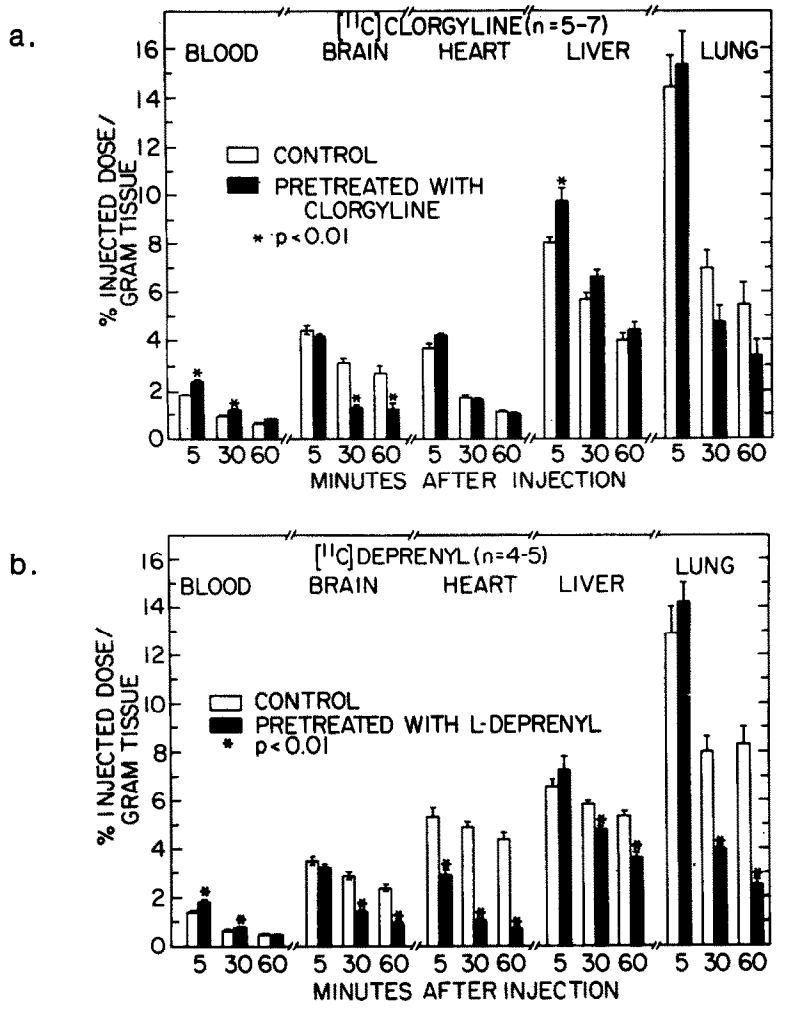

Fig. 1. Time course of distribution of radioactivity in mice (average ± S.E.M. of four to seven animals) following administration of (a) [^{11}C]clorgyline or (b) [^{11}C]-L-deprenyl in control and pretreated animals. Asterisks (*) indicate values which differ significantly from control ($p < 0.01$).

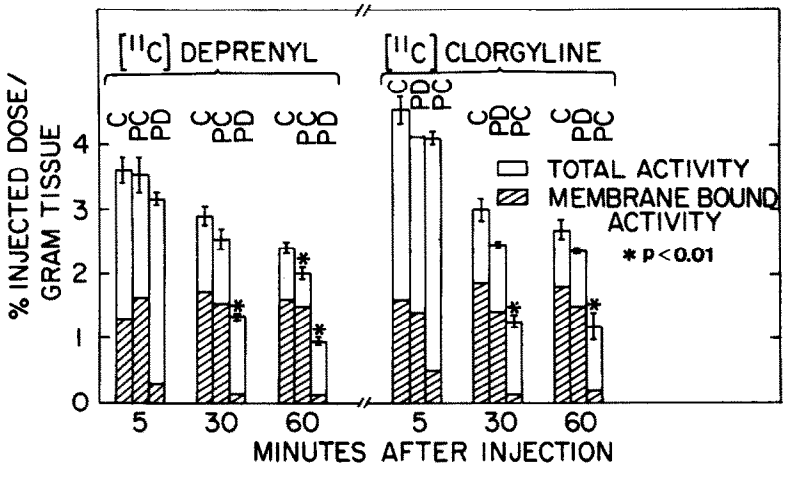

Fig. 2. Time course of radioactivity in brain of control mice (C) and those pretreated with clorgyline (PC) or L-deprenyl (PD). Open bars: Total activity (average ± S.E.M. of four to eight animals). Hatched bars: Activity bound to membranes.

15% of the non-bound radioactivity is still in the form of unmetabolized [^{11}C]MAOI at 30 min.

Thus, these positron emitting tracers may indeed allow the in vivo study of functional MAO A and B in humans and animals utilizing PET. Work is currently underway to assay MAO A and B activities of the various organs of mice to correlate with the differential uptake and clearances of [^{11}C]MAOIs and also to correlate the decrease in specific membrane binding to the degree of inhibition of the MAO subtypes by assaying for residual MAO A and B activities in pretreated animals. The determination of the kinetics of the in vivo metabolism of [^{11}C]MAOIs as part of developing a tracer kinetic model is also in progress. PET studies using baboons as a primate model will form the basis for extending this general approach to measuring in vivo functional enzyme activity in the living human brain and other organs.

In summary, the results of this preliminary study utilizing pretreatment and cross pretreatment experiments strongly support the premise of specific binding in vivo of [^{11}C]clorgyline to MAO A and [^{11}C]-L-deprenyl to MAO B.

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