Imaging beta-adrenoceptors in the human brain with (S)-1'-[F-18]fluorocarazolol

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We evaluated the suitability of fluorocarazolol for in vivo studies of cerebral beta-adrenoceptors because (S)-1'-[18F]fluorocarazolol has a higher affinity to beta-adrenoceptors than to serotonin receptors (pK\textsubscript{A}, 9.4, \beta\textsubscript{2} 10.0, 5HT\textsubscript{1A} 7.4, 5HT\textsubscript{1B} 8.1) and rapidly crosses the blood-brain barrier. Methods: The (S)-[18F]fluorocarazolol (74 MBq, >37 TBq/mmol) was intravenously administered to healthy volunteers on two separate occasions with an interval of at least 1 wk. The initial injection was without pretreatment, but before the second injection, the volunteers received the beta blocker (\dagger)pindolol (3 x 5 mg orally, during 18 hr). The brain was studied with PET. Results: Uptake of radioactivity delineated gray matter and was particularly high in the posterior cingulate, precuneus and striatum. Low uptake occurred in the cingulate, precuneus and striatum. High uptake was significantly reduced in the posterior cingulate and precuneus, and uptake in the striatum was relatively high. Conclusions: Fluorocarazolol is the first radioligand that can visualize cerebral beta-adrenoceptors and may enable monitoring of these binding sites during disease.

Key Words: beta-adrenoceptors; brain; PET; fluorine-18-fluorocarazolol

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An intriguing problem in biomedical research is that of relating symptoms of neurological as well as psychiatric distur-

bances to altered neurotransmitter binding in distinct regions of the brain. Cerebral beta-adrenergic binding sites for the neurotransmitter noradrenaline have been reported to be affected in a variety of disorders, such as depression (1,2), schizophrenia (3), alcoholism (4), Alzheimer’s disease (5) and Huntington’s chorea (6). They appear to play a role in many physiological and behavioral responses, such as glial proliferation (7,8), control of respiration (9), processing of visual information (10), memory function (11) and adaptation to stress (12). The generally observed delayed onset of action of antidepressant drugs may occur because downregulation of beta-adrenoceptors and serotonin receptors takes place only after chronic drug administration (13,14). Receptor downregulation could be a prerequisite of the antidepressant activity (15). Receptor density may also change when noradrenergic innervation is impaired. Deterioration of noradrenergic neurons occurs such as in the Parkinson dementia complex (16).

Most of these observations have been made in autopsy studies, in binding assays to human lymphocytes and cultured cells or in animal experiments. None of these relationships has been observed in intact humans by external detection with receptor-specific radioligands. Fluorocarazolol, a fluorinated analog of the potent beta-blocker carazolol has been useful in vivo studies of beta-adrenoceptors, both in experimental animals (17-19) and in humans (20). Uptake in the rat brain is saturable, sensitive to selective beta-adrenoceptor antagonists and stereospecific. We now report the first results obtained with (S)-1'-[18F]fluorocarazolol-PET to visualize the distribution of beta-adrenoceptors in the brain of healthy volunteers.
MATERIALS AND METHODS

Radioligand

(S)-Desisopropylcarazolol (enantiomeric excess >98%) was prepared as reported previously (17). (S)-1'-[18F]fluorocarazolol was synthesized by reacting the precursor with [18F]fluoroacetone (17,19) and purified by HPLC. The specific activity was 75 ± 35 TBq/mol (2040 ± 950 Ci/mol) and the radiochemical purity was >99.8%. The ligand was dissolved in 0.5 ml ethanol/pro-pylene glycol/0.9% NaCl (1/2/2 v/v/v). Before injection, this solution was filtered (0.22 μm) and 7.5 ml 0.9% NaCl were added through the filter. The solution was sterile and pyrogenic. (S)-1'-fluorocarazolol. HC1 passed the test on acute toxicity (European Pharmacopeia; Dutch Pharmacopeia Ed. IX) at a 10,000-fold higher dose than was administered to humans.

In Vitro Binding Assays

The affinity of (S)-1'-fluorocarazolol to β1- and β2-adrenoceptors was assessed in membranes prepared from human right atrial tissue and human lymphocytes, respectively, using (-)-[18F]fluorocarazolol as the radioligand and the affinity to 5HT1B receptors was determined in rat striatal membranes, using 150 pM [125I]iodocyanopindolol in the presence of 60 μM (-) isoproterenol. The resulting IC50 values were converted to Kd values according to the Cheng and Prusoff equation (22): $K_d = IC_{50}/(50%) = K_{d}$, where IC50 = concentration of fluorocarazolol that inhibited radioligand binding by 50%, [S] = concentration of radioligand in the assay and Kd = equilibrium dissociation constant of the radioligand. The β1- and β2-adrenoceptor assays were performed by the Institut für Pharmakologie und Toxikologie, Halle, Germany; affinities to 5HT1A and 5HT1B receptors were determined by NovaScreen (Hanover, MD).

Human Volunteers

Healthy volunteers were recruited through advertisements in a local newspaper. Excluded were people with: (a) a positive history regarding neuropsychiatric disease; (b) use of antidepressants, beta-blockers, beta-mimetics or theophylline; (c) high blood pressure or heart failure; or (d) pregnancy or suspected pregnancy. All volunteers regarded neuropsychiatric disease. (b) use of antidepressants, beta-blockers, beta-mimetics or theophylline; (c) high blood pressure or heart failure; or (d) pregnancy or suspected pregnancy. All subjects were informed about the purpose and hazards of the experiment both orally and in writing and gave informed consent.

Study Protocol

At the beginning of the study, a cannula was placed in a vein of one of the lower forearms. Another cannula was placed in a radial artery of the contralateral arm after patency of the ulnar artery had been proven by the Allen test. The arterial cannula was inserted under local anesthesia with lidocain. The venous cannula was used for injection of the ligand, the arterial line for blood sampling. Tracer injection, data acquisition and sampling were performed as on day one.

Data Analysis

ROIs were drawn by hand on white matter (corpus callosum), posterior part of the gyrus cinguli including precuneus, striatum, thalamus and cerebral cortex (comprising both hemispheres in a section just superior to the border of white and gray matter). Time-activity curves for the ROIs were calculated using ECAT software (version 6.5D) running on a Sun/Sparc (Mountain View, CA) workstation. All data were normalized to an injected radioactive activity of 74 MBq (2 mCi) and a body weight of 70 kg. The time-activity data were exported to an IBM-compatible PC and characterized using a nonlinear regression data analysis program (EnzFitter, Elsevier Biosoft, Cambridge, U.K.). Differences between groups were tested using one-way analysis of variance and appropriate software (Statistix, NH Analytical, Roseville, MN). A two-tailed probability smaller than 0.05 was considered statistically significant.

RESULTS

General

Preliminary in vitro assays showed that (S)-1'-fluorocarazolol binds preferentially to beta-adrenoceptors (pKd at the β1-subtype: 9.4, at the β2-subtype: 10.0) and has less affinity to 5HT1A (pKd 7.4) and 5HT1B (pKd 8.1) serotonergic sites. These results encouraged us to initiate the pilot study in healthy volunteers.

Details regarding the participants and the study protocol are presented in Table 1. None of the volunteers showed any symptoms of heart failure, hypertension or neuropsychiatric disease.

Distribution of Radioactivity within the Brain

The cerebral distribution of the flow tracer, H215O, delineated gray matter as expected. Representative images acquired after injection of S-1'-[18F]fluorocarazolol are presented in Figure 1. Transaxial cross-sections in the time frame 14–60 min are...
displayed. In the initial study (without pindolol), gray matter was clearly demarcated from white matter. Uptake of radioactivity was especially high in the cortical areas (posterior cingulate, precuneus) and the corpus striatum (caudate and putamen, Fig. 1). Low uptake occurred in the thalamus. The lowest uptake was observed in the white matter (corpus callosum, see Fig. 1). This relatively high striatal uptake and low uptake in the thalamus contrasted with the more even distribution of radioactivity seen in the H215O scans. In one volunteer, a hot spot was visible just ventrally to the cerebellum and interpreted as the putative locus coeruleus (see Fig. 1). After ingestion of pindolol, cerebral uptake of radioactivity was strongly (>two-fold) suppressed and gray matter was no longer demarcated, as the distribution of radioactivity became virtually homogeneous throughout the brain (see Fig. 1 and Table 2). The hot spot near the cerebellum also disappeared after preloading of the volunteer with pindolol.

**Kinetics of Cerebral (5)-1'-[18F]Fluorocarazolol Uptake**

After injection of the radioligand, cortical levels of radioactivity rapidly rose to a maximum followed by an equally rapid, small decline to a relatively stable plateau that was reached within 3 min (Fig. 2). Pindolol accelerated the washout of radioactivity from the cortex (Fig. 2) and it reduced cortical radioactivity to <50% of the control at 60 min postinjection. In contrast to the data for cerebral cortex, uptake of radioactivity in the white matter of the corpus callosum was not significantly affected by pindolol (Fig. 2). Uptake in the corpus callosum was about equal to that in the cortex of pindolol-treated subjects at >30 min postinjection (Fig. 2).

If uptake of radioactivity in the presence of pindolol is considered to represent nonspecific binding, ratios of total-to-nonspecific binding can be calculated. In the cerebral cortex, this parameter increased during the whole period of PET scanning, and it was not yet maximal after 60 min (Fig. 3). The curve fitted to the data suggests that a maximum of 2.52 is reached with a first-order rate constant of 0.0222 min⁻¹ (i.e., time required to reach 50% of the maximum = 31 min).

Washout of radioactivity from the brain of untreated subjects is slow and it appears to be different in different brain areas (faster in thalamus than in other areas of the brain). The relative amount of radioactivity which remains in different areas at 60

### Table 1

<table>
<thead>
<tr>
<th>Date</th>
<th>Volunteer no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Pre-treatment</th>
<th>Injected mass (nmol)</th>
<th>Scan type</th>
</tr>
</thead>
<tbody>
<tr>
<td>05-24-95</td>
<td>1</td>
<td>36</td>
<td>M</td>
<td>67</td>
<td>None</td>
<td>0.88</td>
<td>Static</td>
</tr>
<tr>
<td>05-31-95</td>
<td>1</td>
<td>55</td>
<td>M</td>
<td>82</td>
<td>Pindolol</td>
<td>1.01</td>
<td>Static</td>
</tr>
<tr>
<td>07-12-95</td>
<td>2</td>
<td>27</td>
<td>F</td>
<td>56</td>
<td>None</td>
<td>0.67</td>
<td>Dynamic</td>
</tr>
<tr>
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<td>27</td>
<td>F</td>
<td>56</td>
<td>Pindolol</td>
<td>1.75</td>
<td>Static</td>
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<tr>
<td>08-28-95</td>
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<td>F</td>
<td>56</td>
<td>Pindolol</td>
<td>0.59</td>
<td>Static</td>
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<tr>
<td>11-01-95</td>
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<td>72</td>
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<td>0.40</td>
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<tr>
<td>11-08-95</td>
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<td>M</td>
<td>72</td>
<td>Pindolol</td>
<td>0.35</td>
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<tr>
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<td>F</td>
<td>70</td>
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<td>1.33</td>
<td>Static</td>
</tr>
<tr>
<td>01-09-96</td>
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<td>21</td>
<td>M</td>
<td>69</td>
<td>Pindolol</td>
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<tr>
<td>01-17-96</td>
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<td>21</td>
<td>M</td>
<td>69</td>
<td>None</td>
<td>0.24</td>
<td>Dynamic</td>
</tr>
<tr>
<td>02-28-96</td>
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<td>70</td>
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<tr>
<td>02-14-96</td>
<td>8</td>
<td>25</td>
<td>M</td>
<td>70</td>
<td>None</td>
<td>0.27</td>
<td>Dynamic</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td>29 ± 11</td>
<td>70 ± 7</td>
<td></td>
<td></td>
<td></td>
<td>0.79 ± 0.47</td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 1.** PET brain images at the level of the cingulate cortex + precuneus, striatum and locus coeruleus (from left to right). The upper row of images was acquired in the untreated condition; the lower row after the volunteer had ingested pindolol (time frame 14–60 min). The volunteer is on his back; the direction of observation is from his feet towards his head.
TABLE 2
Uptake of Fluorine-18 in Different Brain Areas 20-60 min Postinjection

<table>
<thead>
<tr>
<th>Area</th>
<th>Untreated</th>
<th>Pindolol-preloaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cingulate</td>
<td>14.6 ± 0.8</td>
<td>62 ± 0.5*</td>
</tr>
<tr>
<td>Striatum</td>
<td>14.3 ± 0.8</td>
<td>63 ± 0.6*</td>
</tr>
<tr>
<td>Superficial layers of the cortex</td>
<td>12.9 ± 0.9</td>
<td>62 ± 0.9*</td>
</tr>
<tr>
<td>Thalamus</td>
<td>8.3 ± 0.9</td>
<td>5.6 ± 0.3*</td>
</tr>
<tr>
<td>White matter</td>
<td>6.3 ± 0.4</td>
<td>5.7 ± 0.9</td>
</tr>
</tbody>
</table>

*Significant differences between the untreated and pindolol-preloaded condition (one-way ANOVA P < 0.05).

Tissue uptake of radioactivity is expressed as camera units (ECAT cts/pixel/sec) x 10^4. Data are an average ± s.e.m. of all dynamic studies (five in the control condition, four after pindolol preload); values were normalized to an injected dose of 74 MBq (2 mCi).

min is: cingulate cortex plus precuneus = striatum > other parts of the cortex > thalamus > white matter of the corpus callosum.

Effect of Beta-Blockade on Radioligand Clearance and Metabolism

Preloading of volunteers with pindolol affects clearance and metabolism of the radioligand (Fig. 4). Levels of radioactivity in the circulation at 20–60 min postinjection are higher, and the fraction of plasma radioactivity representing parent compound also is higher after treatment of volunteers with pindolol. Similar results had been obtained in a previous study of beta-adrenoceptors in the human thorax (20).

DISCUSSION
Selectivity of Fluorocarazolol

Many beta-blockers interact with central serotonergic sites (24–26). Iodocyanopindolol (24,26), pindolol (27), carteolol (25) and propranolol (25,27) bind almost equally well to 5HT1A and/or 5HT1B receptors as to beta-adrenoceptors. In contrast, hydrophilic atenolol (25) and CGP-12177 (26) are selective beta-adrenoceptor antagonists. Unfortunately, 11C-atenolol and 11C-CGP 12177 cannot be used for PET studies of the brain as CGP 12177 hardly crosses the blood-brain barrier (28,29), while the affinity of atenolol is too low for successful beta-adrenoceptor imaging (30). Because of its lipophilicity [log P 2.2 at pH 7.4, (19)], fluorocarazolol was expected to show rapid transport over the blood-brain barrier. Indeed, we have observed that (S)-1'-[18F]fluorocarazolol enters rat brain and it demonstrates significant specific binding in cerebral cortex and cerebellum (18). In vitro binding assays have shown a much higher affinity of fluorocarazolol to beta-adrenoceptors than to 5HT1A or 5HT1B receptors (see Results). The 20- to 400-fold selectivity of fluorocarazolol for beta-adrenoceptors indicates that serotonergic sites will not significantly contribute to the specific binding in the brain after administration of a nanomolar dose of the radioligand (see also below).

Distribution of Binding Sites for Fluorocarazolol within the Brain

Whether a ligand binds to receptors in the living human brain can be determined by assessing whether the regional distribution of radioactivity after drug injection parallels the distribution of receptors known from post mortem autoradiography, and by examining whether administration of an excess of an
unlabeled receptor antagonist blocks this specific regional distribution. Both approaches were used in this study.

After administration of (S)-1'-'[18F]fluorocarazolol to volunteers, distribution of radioactivity within the brain was homogeneous. Relatively high uptake was observed in the striatum and cortical areas (especially cingulate cortex and precuneus). Low levels of radioactivity were present in the thalamus and the lowest in white matter. Such a distribution corresponds to the localization of \( \beta \)-adrenoceptors known from post mortem autoradiography with the ligands \([125I]\)iodocyanopindolol, \([125I]\)iodopindolol and \([3H]\)CGP 12177: high \( \beta \)-adrenoceptor densities are found in caudate and putamen (2,13,31–33), moderate to high densities in various cortical areas (2,13,31–33) and low densities in thalamus (2,13,32) and white matter (33,34). With the most selective \( \beta \)-adrenoceptor ligand, \([3H]\)CGP-12177, the following receptor densities were measured: caudate/putamen 100–134, cortex 55–80, thalamus 38–45 and white matter <20 fmol/mg protein (1,2,13,31). If we assume that 10% of tissue wet weight consists of protein, receptor densities in cortex range from 5.5–8.0 pmol/g. The cerebral concentration of fluorocarazolol in this study was <0.03 pmol/ml. Thus, \( \beta \)-adrenoceptor occupancy by fluorocarazolol was negligible (i.e., <0.5%).

In one volunteer (Volunteer 2), the putative locus coeruleus was delineated after injection of (S)-1'-'[18F]fluorocarazolol. The locus coeruleus does not contain a particularly high amount of \( \beta \)-adrenoceptors, but strong nonspecific binding of \([125I]\)iodocyanopindolol has been reported in this region, which is probably related to the presence of neuromelanin (32). The observation that a pindolol preload suppresses uptake of \(^{18}\)F in the pons of Volunteer 2 (Fig. 1) suggests that binding of fluorocarazolol, in contrast to that of iodoypindolol, is not truly nonspecific but takes place to non-\( \beta \)-adrenergic energetic sites as has been reported for iodoypindolol in the heart (35).
Cerebral Sparganosis: Increased Uptake of Technetium-99m-HMPAO

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Cerebral sparganosis is an extremely rare intracranial parasitic infectious disease. We report findings of 99mTc-HMPAO cerebral perfusion SPECT in a case with cerebral sparganosis. SPECT revealed an irregularly shaped area with markedly increased 99mTc-HMPAO uptake in the parasitic infectious region of the cerebrum. Both white and gray matter was involved, the white matter involved predominantly. Decreased perfusion to the right cerebellum, suggesting cross cerebellar diaschisis, was also demonstrated. This article illustrates that cerebral sparganosis is one of the causes of increased 99mTc-HMPAO uptake in the cerebrum and should be considered clinically if present.

Key Words: sparganosis; technetium-99m-HMPAO; cerebral perfusion SPECT

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This article demonstrates the findings of 99mTc-HMPAO cerebral perfusion SPECT in a case with cerebral sparganosis.

CASE REPORT

A 74-yr-old man patient presented to our hospital with seizure and progressive weakness of the right side of his body for 1 mo. The patient did not have a fever. Neurological examination revealed decreased sensation and muscle power of the right side of the body. The white blood cell count on admission was 5,900/mm³, and the differentiation showed 70% granulocytes, 19% lymphocytes, 4% eosinophils and 7% monocytes. EEG revealed continuous, focal, slow waves over the left frontotemporal area. Brain CT revealed a cystic, enhancing mass lesion at left temporal area. Based on the clinical presentations, examinations, CT and MRI findings brain tumor was suspected.

Technetium-99m-HMPAO cerebral perfusion SPECT was arranged to evaluate the regional blood flow to the intracranial mass lesion. SPECT imaging was performed using a triple-head gamma camera equipped with fan-beam collimators. Acquisition was started 20 min after an intravenous injection of 925 MBq (25 mCi) 99mTc-HMPAO in 120 projections, 3° apart, in a 128 × 128 matrix.
Imaging Beta-Adrenoceptors in the Human Brain with (S)-l-[18F]Fluorocarazolol

Aren van Waarde, Ton J. Visser, Philip H. Elsinga, Bauke M. de Jong, Thom W. van der Mark, Jan Kraan, Kees Ensing, Jan Pruim, Antoon T.M. Willemsen, Otto-Erich Brodde, Gerben M. Visser, Anne M.J. Paans and Willem Vaalburg


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