Imaging Neuroreceptors in the Living Human Brain

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ABSTRACT

For nearly a century it has been known that chemical activity accompanies mental activity, but only recently has it been possible to begin to examine its exact nature. Positron-emitting radioactive tracers have made it possible to study the chemistry of the human mind in health and disease, using chiefly cyclotron-produced radionuclides, carbon-11, fluorine-18 and oxygen-15. It is now well established that measurable increases in regional cerebral blood flow, glucose and oxygen metabolism accompany the mental functions of perception, cognition, emotion and motion. On May 25, 1983 the first imaging of a neuroreceptor in the human brain was accomplished with carbon-11 methyl spiperone, a ligand that binds preferentially to dopamine-2 receptors, 80% of which are located in the caudate nucleus and putamen. Quantitative imaging of serotonin-2, opiate, benzodiazapine and muscarinic cholinergic receptors has subsequently been accomplished. In studies of normal men and women, it has been found that dopamine and serotonin receptor activity decreases dramatically with age, such a decrease being more pronounced in men than in women and greater in the case of dopamine receptors than serotonin-2 receptors. Preliminary studies in patients with neuropsychiatric disorders suggests that dopamine-2 receptor activity is diminished in the caudate nucleus of patients with Huntington's disease. Positron tomography permits quantitative assay of picomolar quantities of neuro-receptors within the living human brain. Studies of patients with Parkinson's disease, Alzheimer's disease, depression, anxiety, schizophrenia, acute and chronic pain states and drug addiction are now in progress.

The growth of any scientific field is based on a paradigm or set of ideas that the community of scientists accepts. The unifying principle of nuclear medicine is the tracer principle applied to the study of human disease. Nineteen hundred and sixty-three was a landmark year in which technetium-99m and the Anger camera combined to move the field from its latent stage into a second stage characterized by exponential growth within the framework of the paradigm. The third stage, characterized by gradually declining growth, began in 1973. Faced with competing

advances, such as computed tomography and ultrasonography, proponents and participants in the field of nuclear medicine began to search for greener pastures or to pursue narrow subspecialties. Research became characterized by refinements of existing techniques.

In 1983 nuclear medicine experienced what could be a profound change. A new paradigm was born when it was demonstrated that, despite their extremely low chemical concentrations, in the picomolar range, it was possible to image and quantify the distribution of receptors in the human body. Thus, nuclear medicine was able to move beyond physiology into biochemistry and pharmacology.

Fundamental to the science of pharmacology is the concept that many drugs and endogenous substances, such as neurotransmitters, react with specific macromolecules that mediate their pharmacologic actions.

Such receptors are usually identified in the study of excised tissues, cells or cell membranes, or in autoradiographic studies in animals. The first imaging and quantification of a neuroreceptor in a living human being was performed on May 25, 1983 and reported in the September 23, 1983 issue of SCIENCE. The study involved the development and use of carbon-11 N-methyl spiperone (NMSP), a drug with a high affinity for dopamine receptors. Since then, studies of dopamine and serotonin receptors have been carried out in over 100 normal persons or patients with various neuropsychiatric disorders. Exactly one year later, the first imaging of opitate receptors in a living human being was performed [1].

C-11 Methyl Spiperone

In vitro binding studies indicate that NMSP has a high affinity for D2 dopamine receptors and an approximately 5-fold less affinity for S2 serotonin receptors. In the human brain, D2 dopamine receptors are most plentiful in the caudate and putamen while S2 serotonin receptors are greater in number in cerebral cortical areas.

In vivo drug competition studies in rodents revealed that 90% of the accumulated [^{11}D] NMSP in the striatum was to D2 receptors while the majority of the binding in the frontal cortex was to S2 receptors. The nonspecific binding is estimated by the level observed in the cerebellum. In vivo PET studies with baboons, specific [^{11}C]NMSP binding in the caudate was blocked by competition with excess unlabeled spiperone. The [^{11}C]NMSP accumulation, when corrected for nonspecific binding, in basal ganglia reflects binding to D2 dopamine receptors while the majority of accumulation in cerebral cortical areas reflects binding to S2 serotonin receptors.

Effects of Age and Sex on Dopamine and Serotonin Receptors

The 22 male and 22 female volunteers ranging in age from 19 to 73 years, and 19-67 years, respectively (males: mean age 39 + 17; females: mean age 36 + 14 [+1 standard deviation]) were found to be healthy by medical, neurological, and neuropsychological tests. CT scans were taken of each subject to identify planes which contained the basal ganglia. Subjects were injected

with [^{11}C]NMSP (dose range was 0.2 – 2.9 ug/kg) which was synthesized by the N-alkylation of spiperone with [^{11}C] methyliodide. Multiple, serial PET scans were obtained over a two-hour imaging time after injection. The distribution of tracer in the brain within the first few minutes after injection is determined principally by regional blood flow. D2 dopamine receptor binding density was estimated by the ratio of the caudate to the cerebellar (Ca/Cb) mean counts/pixel (computer matrix picture element) and the putamen to cerebellar mean counts per pixel (Pu/Cb). S2 serotonin receptor binding was estimated by frontal cortex to cerebellar ratios.

After injection, accumulation of [^{11}C]NMSP within the caudate and putamen, areas rich in D2 receptors, increased progressively in time while binding in the cerebellum which contains few or no D2 receptors, decreased steadily after its initial accumulation related to regional blood flow. Binding in the frontal, temporal, parietal and occipital cortex decreased at an intermediate rate, reflecting primarily S2 receptor binding and decreasing nonspecific binding (Fig. 1). The ratio of Ca/Cb increased linearly with time (Fig. 2). The slope of this line reflects the rate (sometimes referred to as k_3) of NMSP binding to the D2 receptor from the exchangeable tissue pool (including nonspecifically bound).

A plot of Ca/Cb versus age revealed a striking decline in D2 receptor binding with age. In males, the data were best fit by an exponential function [Ca/Cb = 2.31 + 6.6 exp (−0.06 age);

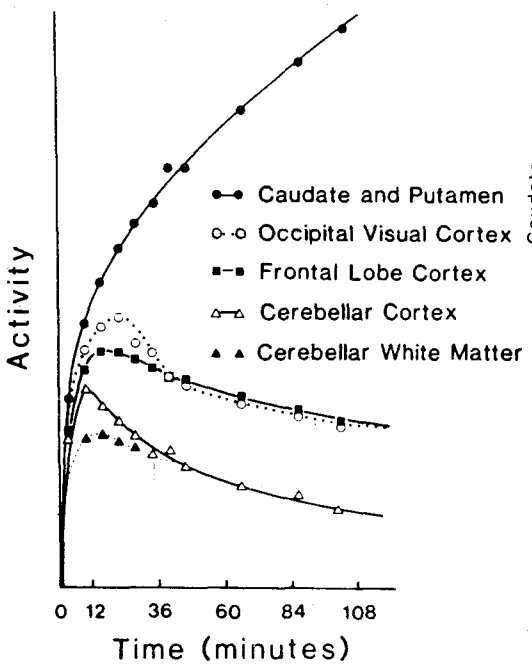

Fig. 1. Time course of activity measured in various parts of the normal human brain after intravenous injection of carbon-11 N-methyl spiperone.

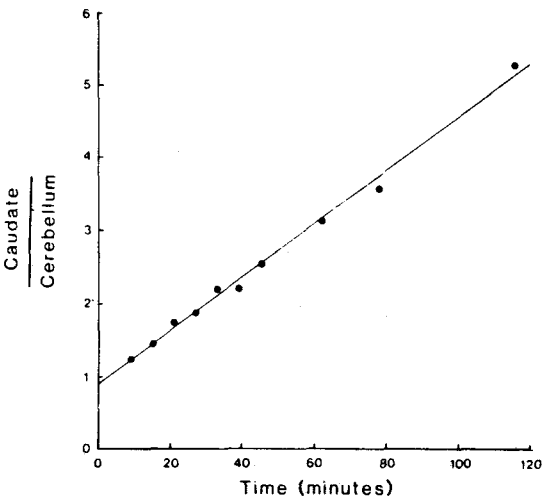

Fig. 2. The ratio of caudate/cerebellar activity as a function of time after intravenous injection. The caudate activity represents specific binding of the carbon-11 methyl spiperone to dopamine-2 receptors, while the cerebellar activity represents non-specific binding. The slope of the line reflects the net binding of the tracer to dopamine-2 receptors.

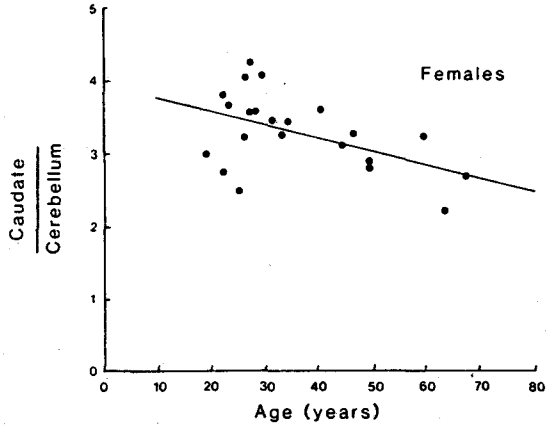

Fig. 3. Decrease in dopamine-2 receptor activity (caudate/cerebellar ratio) as a function of age in normal men.

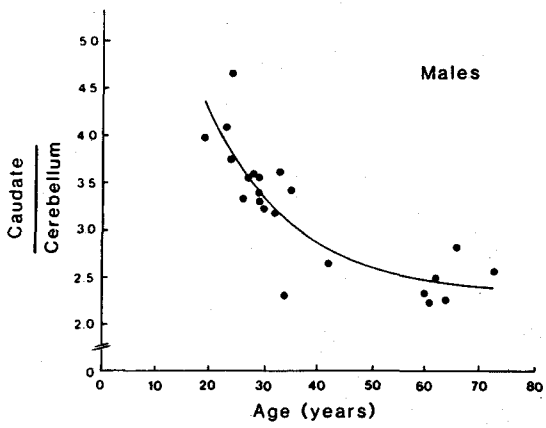

Fig. 4. Decrease in dopamine-2 receptor activity as a function of age in normal women. In men the decrease was best fitted by an exponential function; in women, the decrease was linear.

R = 0.86]. There was a 46% decline in the fitted function over the age range (19-73 years) examined (Fig. 3-4). There was a similar 43% decline in the putamen [Pu/Cb = 2.4 + 6.37 exp (−0.065 age); R = 0.81], another region with high levels of D2 receptors, with age (not shown). In the 22 women there was a somewhat smaller, 25%, decrease in receptor binding with age (19-67 years) in the caudate (Ca/Cb = 3.97 − 0.0185 age; R = 0.58; Fig. 3) and putamen (R = 0.6). As mentioned above, a plot of Ca/Cb against time was a straight line for any subject (Fig. 2). When the slopes of the lines were a plotted against age for males, an exponential decline was observed.

In the frontal cortex, a region containing primarily S2 receptors, there was also a significant drop in binding with age. A plot of the ratio of frontal cortex to cerebellum, an estimate of S2 serotonin receptors, against age revealed a decline with age in males [ratio = 1.35 + 1.75 exp (−0.053 age); R = 0.7], (Fig. 5). Again, there was a smaller decline in females (ratio = 1.86 − 0.0066 age; R = 0.4).

In vitro studies with both animal and human tissues at autopsy have revealed a decline in D2 receptor B_{max} with age with no change in K_D. A decline in cerebral blood flow alone does not seem to be adequate to explain the findings, since the decline of blood flow with age is much less than the decline in receptor binding. With NMSP, the initial 0-6 min after injection distribution is predominantly determined by blood flow. There was no significant decline in the caudate/cerebellar ratio (approximately 1) obtained from the 0-6 min scans as a function of age in either the men or women. The data indicate that there is no fall in early (0-6 min) caudate to cerebellar ratios with age ($p > 0.05$) for either sex. In addition, the ratio of the caudate counts (0-6 min) to the integral of the activity in the blood, and the ratio of the cerebellum counts to the integral of the blood activity did not fall with age for either sex. All CT scans of normal volunteers were reviewed to assess whether the caudate nucleus or putamen decreased in size with age. The CT

scans did not reveal any trend for a decrease in size and therefore the decline in the ratios are not due mainly to changes in partial volume effects. Cerebral glucose metabolism, also measured by PET, has been found by most investigators either not to decrease, or to drop only at very slow rates with age.

Other studies have revealed age-related decreases in various other dopaminergic parameters [2-9]. Cell bodies in the substantia nigra and putamen decrease in number and size with age. There is also an age-related decrease in the levels of neurotransmitter as well as in levels of synthesizing enzymes in the caudate, putamen and nucleus accumbens. These regions have high concentrations of dopaminergic nerve terminals and receptors.

Figures 6 and 7 are typical images obtained at three different levels of the brain of a normal person (Fig. 6) and a patient with a stroke involving the left middle cerebral artery.

Opiate Receptors

Imaging of opiate receptors was accomplished by developing 4-carbo-methoxy-fentanyl, also called carfentanil. This compound is an extremely potent agonist of the opiate receptor, over 7000 times more potent than morphine. The synthesis time was 35 minutes from the end of bombardment in the cyclotron. The average specific activity in 10 consecutive syntheses was 1145 mCi/micromole at the end of synthesis. This corresponds to an average specific activity at the end of bombardment of 3270 mCi/micromole.

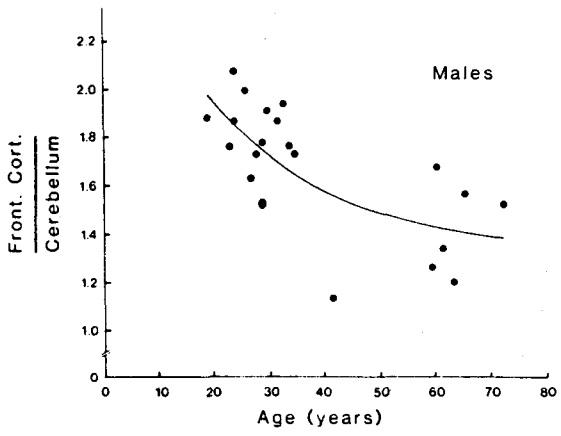

Fig. 5. Decrease in serotonin-2 receptor activity (frontal cortex/cerebellar activity) in normal men as a function of age.

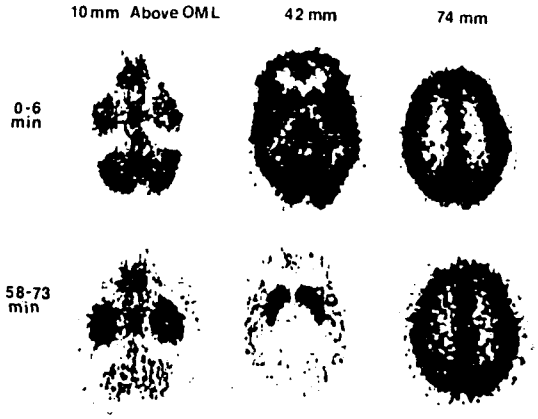

Fig. 6. Images of carbon-11 methyl spiperone distribution at three levels of the brain of a normal person at the times indicated after intravenous injection. The 0-6 images reflect the distribution of blood flow; the 58-73 minute images represent the distribution of dopamine-2 and serotonin-2 receptors.

Carfentanil binds chiefly to mu receptors that are involved in pain perception. The subtype specificity of carfentanil was ascertained by measuring its effect on receptor binding of H 3 dihydromorphine, a mu selective ligand, as well as other ligands that have relatively high specificity for delta and kappa receptors. The calculated affinity constants (K_I) of carfentanil for mu, delta and kappa opiate receptors at 37°C are 0.051 nM, 4.7 nM and 13 nM. When C-11 carfentanil was administered to intact mice, the thalamus and corpus striatum, which are rich in opiate receptors, bound five times higher concentrations of C-11 carfentanil than did the cerebellum. In baboon studies, highest concentrations of radioactivity were found in the thalamus and caudate nucleus, with intermediate concentrations in the frontal cerebral cortex and a negligible concentration in the cerebellum. Pretreatment with naloxone resulted in a markedly reduced accumulation of the tracer in the thalamus, caudate nucleus and cerebral cortex, but not in the cerebellum or soft tissues.

On May 25, 1984, exactly one year after the first images were obtained of dopamine receptors in the living human brain, C-11 carfentanil was administered alone and following naloxone (1 mg/kg intravenously to one of us [HNW]). Images were obtained from 0-3, 10-25 and 30-60 minutes after injection (Fig. 8). In the last image, redioactivity was most highly concentrated in the medial thalamus with substantially lesser levels in the lateral thalamus. The caudate nucleus and putamen displayed a similar high level of radioactivity. The frontal and temporal-parietal

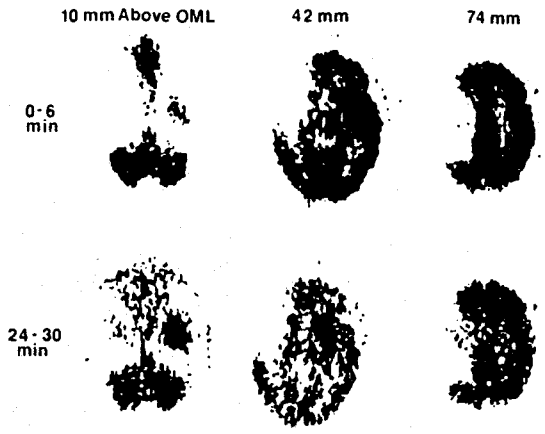

Fig. 7. Images of the distribution of carbon-11 methyl spiperone at the times indicated in a patient with a stroke involving the left middle cerebral artery distribution.

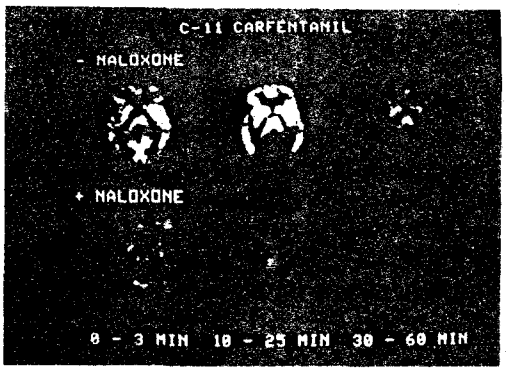

Fig. 8. Images of the distribution of carbon-11 carfentanil in the brain of a normal person at the times indicated after intravenous injection of the tracer. The upper row is the initial study with carfentanil alone; the lower row represents the distribution of a second dose of carbon-11 carfentanil injected five minutes after a blocking dose of naloxone. The initial images reflect predominantly blood flow; the later images the binding of the tracer to opiate receptors.

cerebral cortex had somewhat less radioactivity but substantially more than the occipital cortex. A high level of tracer accumulation was also observed in the pituitary region and temporal lobes of the cerebral cortex while the cerebellum had much lower levels of radioactivity. The post-central gyrus also seemed to accumulate low quantities of the tracer. In the images obtained after the blocking dose of naloxone, the initial 0-3 minute image revealed a distribution of tracer activity that corresponded to the known distribution of regional blood flow. The subsequent images indicated that the uptake by opiate receptors was essentially eliminated by the prior administration of naloxone.

Opiate receptor activity can now be measured in conditions such as depression, mania, Parkinson's disease, schizophrenia, senile dementia, pain states and persons suffering from opiate addiction.

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