RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

An Iodinated Catecholamine Congener for Brain Imaging and Metabolic Studies

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The iodinated O-methylated catecholamine congener, 4-iodo-2,5-dimethoxyphenylisopropylamine (4-I-DPIA), has potential as a new agent for imaging and metabolic studies of the brain and lung. The organ distribution and brain uptake of radioiodine-labeled 4-I-DPIA were studied in the dog and monkey by whole-body scanning, gamma-camera scintigraphy, and organ assay. The brain takes up 2% of the injected dose, with a half-time of 8 sec in the monkey, and the lung takes up 11.8%. An unusual finding was a concentration in the retina, five times that in any other CNS tissue. 4-I-DPIA may have potential in the imaging of normal brain tissues and thereby delineating nonfunctional areas damaged by infarction, trauma, or malignancy, and may also be useful in metabolic studies of catecholamine function. Adequate radioactivity can theoretically be administered with a quantity of 4-I-DPIA $1/10{,}000$ of the pharmacologically active levels. The agent may also find application in lung imaging because of the high pulmonary uptake.

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In previous studies a psychodysleptic compound, 4-^{82}Br-2, 5-dimethoxyphenylisopropylamine (4-Br-DPIA, Fig. 1e), was found to concentrate in brain (*1*), and it was suggested that this compound might find use in clinical nuclear medicine (*2*). The available isotopes of bromine, however, emit high-energy gamma photons that are difficult to collimate, so we decided to investigate the iodine analog of this catecholamine congener, 4-iodo-2, 5-dimethoxyphenylisopropylamine (4-I-DPIA, Fig. 1f). It is given the acronym 4-I-DPIA, in reference to the compound upon which it is based, 2,4,5-trimethoxyphenylisopropylamine (TMA-2, Fig. 1c), in which the 4 substitution position has lost an oxygen atom and gained an iodine atom. We have also developed a rapid synthetic method appropriate for use with I-123, this being a more suitable isotope than I-131 because of the 50-fold improvement in the ratio of useful gammas to tissue radiation dose.

4-I-DPIA is of interest for two reasons.

1. It is the first clinically useful agent that concentrates in normal brain tissue. 4-Br-DPIA concentrates in brain, but the gamma emission of readily available bromine isotopes is of too high an energy for good resolution. Diphenylhydantoin also concentrates in brain and has been labeled with Carbon-11, but the 20-min half-life and the requisite rapid synthetic chemistry limit potential usefulness to laboratories in

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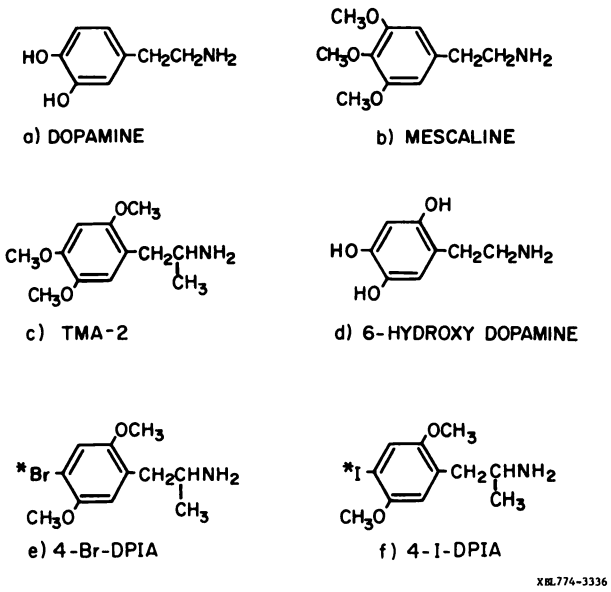

FIG. 1. Structural formulae of 4-I-DPIA and related catecholamine congeners, showing structural similarities.

close proximity to a cyclotron and an organic hot lab. 4-I-DPIA could readily be made with either I-131 or I-123 by radiopharmaceutical laboratories and shipped for use.

2. 4-I-DPIA is structurally related to dopamine (Fig. 1a), 6-hydroxydopamine (Fig. 1d), amphetamine, and psychotomimetic agents such as mescaline (Fig. 1b) and TMA-2 (Fig. 1c), all of which have been associated with a postulated metabolic basis for schizophrenia (*3*). As we shall show in this report, 4-I-DPIA concentrates in the retina, which suggests retinal involvement in hallucinogenesis. Study of the in-vivo kinetics of this and related compounds may provide insight into the metabolic basis of brain disorders that have been associated with the catecholamine neurotransmitters.

METHODS

The synthesis of [^{131}I] 4-I-DPIA was achieved in three steps from 2,5-dimethoxy phenylisopropylamine (*4*). This base was converted to the phthalimide derivative by interaction with an equivalent quantity of phthalic anhyride in refluxing toluene. The isolated imide reacted readily in acetic acid with I-131 monochloride, which was generated by exchange of I-131 ion with nonradioactive ICl, to form the iodinated intermediate N-[2-(2,5-dimethoxy-4-iodophenyl)-1-methylethyl]-phthalimide. This was cleaved to 4-I-DPIA without isolation using hydrazine hydrate in ethanol. The overall yield was 43%. This synthetic route was suitable for I-131, but in order to use 13-hr I-123, modifications were made in the synthesis to reduce the total synthesis time to less than 4 hr (*5*). An especially pure form of I-123, free of contaminating iodine isotopes, was obtained from the Crocker Nuclear Laboratory of the University of California at Davis. Radionuclide dosages were measured with a radionuclide calibrator.

Two female beagle dogs under pentobarbital anesthesia (12.5 kg each) were injected intravenously with [^{131}I] 4-I-DPIA, specific activity 5.9 mCi/mM. Both animals were studied by repeated scans on the Anger Mark 2 whole-body scanner (*6*) which has been digitized (*7*). Dog 1 was prepared for axial tomography 4 hr after injection, and Dog 2 was killed (Euthanol) for organ analysis after 3 hr. Organ distribution studies were done with the whole-body scanner.

Transverse image sections of the head of Dog 1 were generated from multiple views by data reduction, using the Anger camera. The animal was placed in a styrofoam holder and rotated to 36 views. The data for each view were digitized on 64 × 64 frames, and each frame corresponding to various levels in the animal was used as the input projection for transverse-section reconstructions. An iterative least-squares algorithm was used for reconstruction with an attenuation compensation technique that assumes a uniform attenuation coefficient (*8*).

Excised organs from the dog were counted by

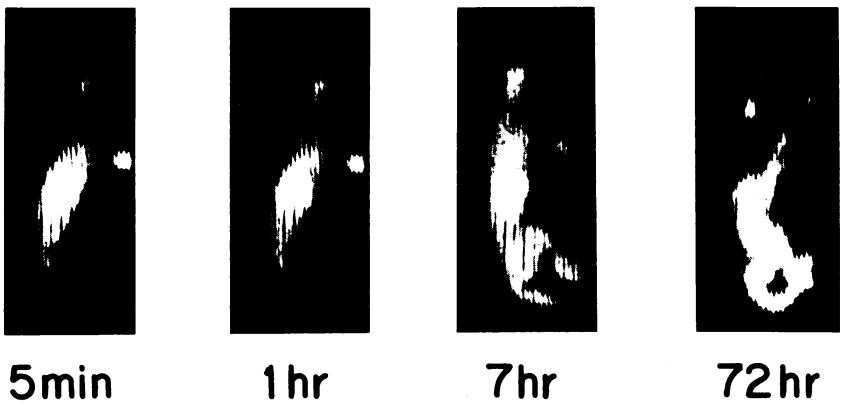

FIG. 2. Whole-body scans of Dog 1 at indicated times after injection of [^{131}I] 4-I-DPIA.

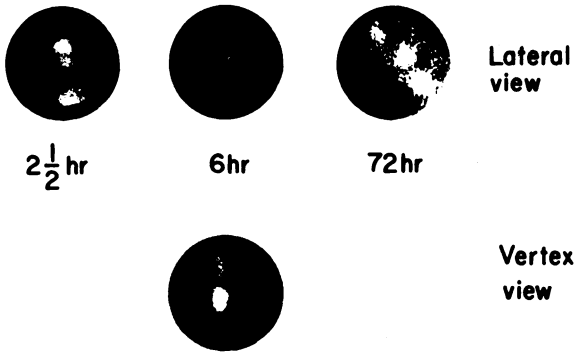

FIG. 3. Scintillation-camera pictures of head of Dog 1. The 2½- and 72-hr pictures were taken with 11-in.-crystal camera with medium-energy collimator, 6-hr pictures (lateral and vertex views) were taken with 14-in.-crystal camera and medium-energy collimator used for tomographic reconstruction.

three different methods. First, the major organs were laid out on the whole-body scanner and counts obtained by designating areas of interest corresponding to the various organs. Samples from these organs (particularly of the brain) and all of the small organs were then placed in tared vials for counting in a well scintillation counter to obtain cpm/gm of the weighed tissue. From these data and an aliquoted standard referred to the injected dose, measured in the radionuclide calibrator, we determined the percentage of the injected dose/gm tissue. While the large-organ analysis is not as accurate as homogenizing each organ and counting weighed aliquots, it provides a simple and reasonably accurate estimate of whole-organ content. The data from the well counter are more reliable because the tissue path length is small and fairly uniform and the counting efficiency is high. The brain was removed from the calvarium and sliced into transverse sections approximately 1 cm thick for imaging with the scintillation camera.

Under pentobarbital anesthesia, a 7.5 kg male Bonnet monkey (*Macaca radiata*) was injected with 3.5 mCi [^{123}I]-4-DPIA with his head under a scintillation camera for determination of the kinetics of brain uptake, using a digitized computer system (7). Subsequent to the uptake measurement, scintiphotos were made from a variety of aspects.

RESULTS

Sequential scans of Dog 1, injected with 1.5 mCi of [^{131}I] 4-I-DPIA, are shown in Fig. 2. The relative organ distribution was found to be stable within 5 min of the injection (the time of the first scan), and did not change appreciably, except for accumulation in the brain, over the succeeding 7 hr. The primary accumulation is in the area of the liver and lungs, with lesser concentration in brain. Activity at the injection site was less than 3.5% of the total, and the activity seen in the hind quarters at 72 hr is due to external contamination from urine. A small amount of activity is in the thyroid at that time.

Scintillation-camera views of Dog 1 are shown in Fig. 3. At 2½ hr, activity is seen to be concentrated in brain; at 6 hr some activity is seen also in the thyroid in both the laterial and vertex views. At 72 hr there is virtually no activity remaining in brain, but the eyes, thyroid, and parotid glands all contain the tracer.

Four hours after injection, the rotational viewing of the head of Dog 1 was begun, to obtain data for axial reconstruction tomography in order to evaluate any preferential localization in the brain. Figure 4 shows the results of four reconstructions of successive

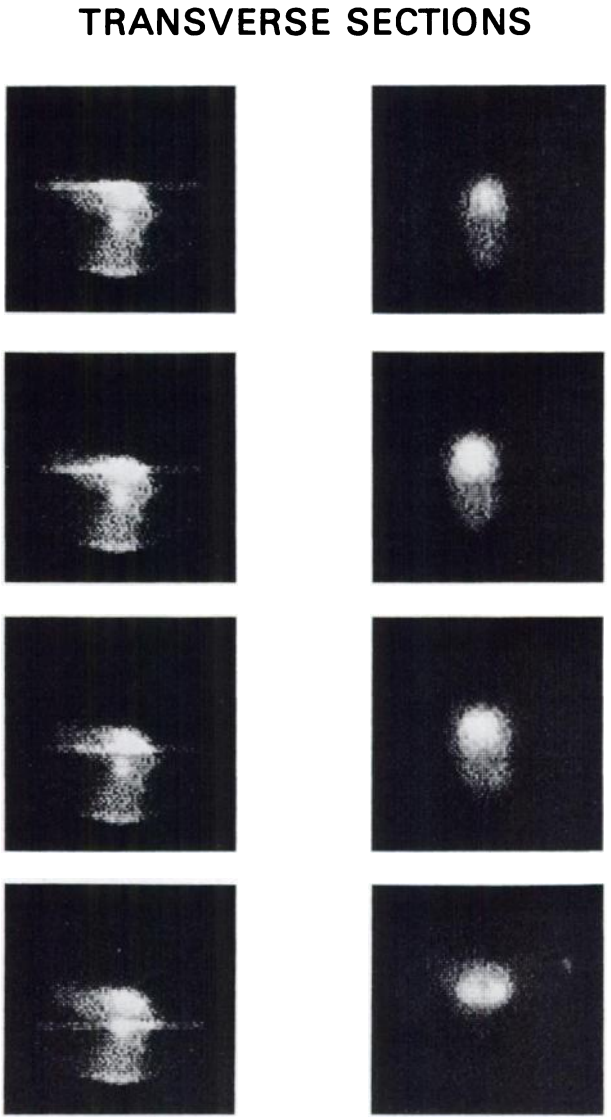

FIG. 4. Computer-reconstructed tomographic images of Dog 1. Left-hand column of pictures shows left lateral views of head with position of tomographic slice indicated by intensified band. Right-hand column shows top views of corresponding slices, with snout pointing down.

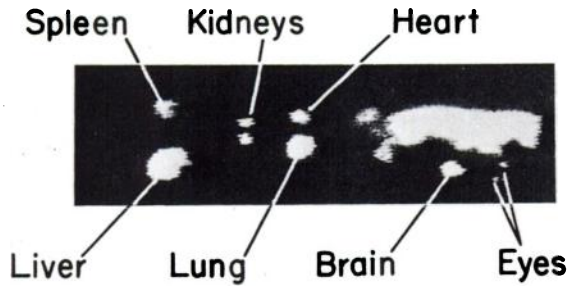

FIG. 5. Postmortem scans of carcass and various organs removed from Dog 2 that were outlined as regions of interest for counting.

horizontal sections of the brain. The area of the brain shows a high concentration of activity compared with the adjacent facial areas, especially the snout. No areas of special concentration are seen within these tomographic slices of the intact living brain. Some concentration is seen at this time in the thyroid, and the two lobes of the thyroid appear clearly in the last section, indicating that any areas of comparable separation and concentration within the brain would have been differentiated had they occurred. The resolution of the reconstructions is about 12 mm FWHM (*9*).

Dog 2 was killed after 3 hr, and the excised organs were imaged with the scanner as shown in Fig. 5. Table 1 shows the total activity in each organ, expressed as percentage of the administered dose, and the concentration as percentage of the dose per gram of tissue. Most of the activity was concentrated in the liver and lungs, with about half of the dose remaining in the carcass. The lung had the highest concentration in percentage dose/gram, but the most striking concentration was in the retina at .098% of the dose per gram—second only to the lung, and twice as high as the liver. Scintillation-camera pictures of the brain slices indicated that no brain area was significantly higher than any other, and this was confirmed by the tissue samples from various brain areas measured in the well counter.

After administration of [^{123}I] 4-I-DPIA in the monkey, the rate of accumulation of I-123 in the brain is rapid, with a half-time of 8 sec. As shown in Fig. 6, the activity persists for hours. The scintiphotos were taken at the times indicated by the uptake curve. Later images, with different views, are also shown.

DISCUSSION

The new radiopharmaceutical 4-iodo-2,5-dimethoxyphenylisopropylamine, 4-I-DPIA, is of interest not only as an agent for positive imaging of normal brain, but also for studies of the mode of action of psychodysleptic drugs and their relation to the metabolic basis of schizophrenia.

The uptake of 4-I-DPIA in the brain of the

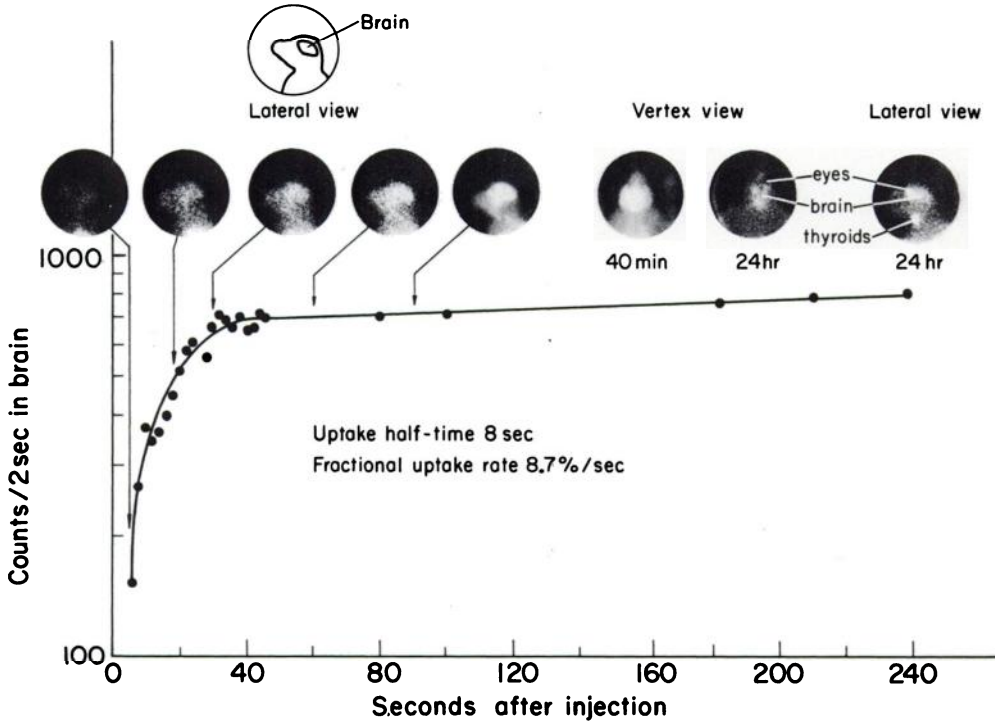

FIG. 6. Brain uptake of I-123 in monkey after administration of [^{123}I] 4-I-DPIA (140-keV collimator). Uptake curve was obtained in 2-sec intervals from area of interest shown in inset. Early pictures are keyed to curve at time they were obtained.

TABLE 1. ORGAN DISTRIBUTION OF I-131 AFTER ADMINISTRATION OF [^{131}I] 4-I-DPIA IN THE DOG*

Organ	% dose / organ	% dose / g
Scanner		
Carcass	52.9	
Gut	10.5	
Lungs	11.8	0.134
Liver	18.7	0.048
Heart	1.1	0.012
Spleen	1.0	0.035
Kidneys	1.2	0.021
Brain	2.0	0.020
Eyes	0.2	0.014
Well counter		
Retina (one)	0.026	0.098
Lacrimal glands	0.0026	0.0013
Adrenals	0.014	0.0052
Ovaries	0.0051	0.0018
Thyroids	0.0099	0.012
Pituitary	0.0005	0.011
Brain tissue:		
caudate nucleus		0.0102
cerebellum, white matter		0.0080
cerebellum, grey matter		0.0109
hypothalamus		0.0089
olfactory and frontal region		0.0103
cerebral cortex, white matter		0.0118

* Obtained by analysis of data from whole-body scanner and scintillation well counts of smaller organs and selected samples of brain tissue.

monkey is complete 40 sec after injection. The distribution in the major organs of the dog is like that found for the bromine congener in man (*1*); liver and lung are the major organs of uptake, with 2% of the dose appearing in brain.

The most unusual finding was the observation of uptake of radioactivity in the eye. Dissection of the retina away from the lacrimal glands and other eye tissues showed clearly that the activity in the retina was ten times that in the surrounding tissues. The concentration of activity in the retina on a per-gram basis was about five times that in other CNS tissues and was exceeded only slightly by that of lung. Activity was also seen in the thyroid and the parotid glands. Iodide is known to be taken up by salivary glands as well as the thyroid, but to our knowledge it has never been found in the retina and does not appear in the eyes on early or late I-131 or I-123 scans. Radioactivity was visible in the thyroid of the monkey at 24 hr and in Dog 1 at 72 hr. Although only .01% of the injected dose was present in the thyroid of Dog 2 at 3 hr after injection, the late appearance and long persistence in the other two animals suggests that this activity represents free iodide resulting from in-vivo metabolism of 4-I-DPIA. In a previous study, less than 5% dehalogenation occurred in-vivo with 4-Br-DPIA (*1*), and it appears that deiodination occurs to an even lesser extent with 4-I-DPIA. The uptake in the retina is not so readily accounted for, and if it represents uptake of the parent 4-I-DPIA molecule, it poses the interesting possibility of a direct relationship between retinal uptake and the visual perceptual disturbances reported for some of the 4-substituted congeners of 4-I-DPIA (*3,10*).

The uptake in the lung removed from Dog 2 is similar to that seen in man with the bromine congener (*1,2*), but the lungs are not as easily visualized in the dog. There has been some interest in the uptake of amines by lung as a mechanism by which the lungs may be imaged with radiopharmaceuticals using carbon-11 aliphatic amines (*11*). If [^{123}I] 4-I-DPIA were found to concentrate suitably in lung for such studies, it would provide a radiopharmaceutical with an ideal gamma energy for scintigraphy and a half-life allowing sufficient time for chemical synthesis and delivery.

There has been interest recently in halogenated dopamine analogs as adrenal-imaging agents (*12*), but no brain or lung uptake was described for these free phenolic dopamine analogs. 4-I-DPIA, a methoxylated analog, has much lower adrenal/kidney and adrenal/liver ratios, indicating that methoxylation has significantly altered the distribution with respect to adrenal uptake.

We have demonstrated that brain is clearly delineated with [^{123}I] 4-I-DPIA in the monkey, and that [^{131}I] 4-I-DPIA can be used to produce reconstructed tomographic image sections. Iodine-123 used in the same way would yield even better images. The synthetic method used (*5*) is applicable to production and purification by TLC of microgram quantities of 4-I-DPIA. The theoretical carrier-free specific activity of [^{123}I] 4-I-DPIA is 770 mCi/μgm, and the pharmacologic dose is of the order of 500 μgm in man, so it would be possible to use it as a scanning agent at levels 1/10,000 of those at which psychopharmacologic effects occur. [^{123}I] 4-I-DPIA should be able to demonstrate ischemic or damaged areas of the brain and thus be useful in locating and measuring the extent of trauma or infarction. It might also find use as a lung-scanning agent. In addition, we feel that a promising potential use of 4-I-DPIA is as an agent for investigating tissue metabolism in the brain.

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