A Rostrocaudal Gradient for Aromatic Acid Decarboxylase in the Human Striatum

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ABSTRACT: The local concentration of 6-[^{18}F]fluoro-L-dopa (^{18}F) reflects the activity of aromatic acid decarboxylase (AADC), the enzyme that generates dopamine from its precursor amino acid, L-dopa. In young healthy adults, the local concentration of ^{18}F, and hence AADC activity, is constant in coronal slices taken in a rostrocaudal direction. With increasing age a gradient representing decreasing activity in the putamen develops. This decrease is less marked than was expected from the literature. In five children with primary dystonia, the striatal distribution of ^{18}F resembled that seen in the normal older adults. In established clinical Parkinson's disease the rostrocaudal gradient becomes steep; the putamen is more damaged.

RÉSUMÉ: Gradient rostro-caudal de l'acide aromatique décarboxylase dans le striatum de l'humain. La concentration locale de 6-[^{18}F]fluoro-L-dopa reflète l'activité de l'acide aromatique-décarboxylase (AAAD), enzyme qui produit la dopamine à partir de son acide aminé précurseur, la L-dopa. Chez les jeunes adultes en bonne santé, la concentration locale de ^{18}F, et donc l'activité de l'AAAD, est constante dans des tranches de section coronales prélevées en direction rostrocaudale. Un gradient représentant une diminution d'activité se développe dans le putamen avec le vieillissement. Cette diminution est moins importante que ce qu'on serait en droit de s'attendre d'après la littérature. Chez 5 enfants atteints de dystonie primaire, la distribution striatale de ^{18}F ressemblait à celle que l'on observe chez des adultes normaux âgés. Dans la maladie de Parkinson cliniquement bien établie, le gradient rostro-caudal prend la forme d'une pente raide; le putamen est plus atteint.

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[^{18}F]fluoro-L-dopa and positron emission tomography have made it possible to study intracerebral dopamine in live humans. The positron emitting radiolabelled molecule 6[^{18}F]fluoro-L-dopa is decarboxylated *in vitro* by L-aromatic acid decarboxylase (EC 4.1.1.26). The Km for this reaction is very similar to that of the reaction when native L-dopa is the substrate.[1] When 6[^{18}F]fluoro-L-dopa is injected i.v. ^{18}F is retained preferentially in the striatum of monkeys and man.[2] It has been assumed that much of this retention has been due to [^{18}F]dopamine stored in the terminals of the nigrostriatal dopaminergic pathway. There is now direct proof that this is the case, at least at early times after the tracer has been given.[3] A series of monkeys was sacrificed at intervals up to 3 hours after being given a single injection of 6[^{18}F]fluoro-L-dopa. Their brains were analysed for their content of the precursor [^{18}F]fluoro-dopa and its metabolic products [^{18}F]dopamine, [^{18}F]homovanillic acid ([^{18}F]HVA), and 0-methyl-[^{18}F]fluoro-dopa. [^{18}F]fluoro-dopa disappeared rapidly from the striatum. [^{18}F]fluoro-dopamine increased both relatively and absolutely to reach a maximum concentration about 20 minutes after [^{18}F]fluoro-dopa had been given. Thereafter its concentration fell. The striatal concentration of [^{18}F]HVA rose gradually during the period of the study and was equal to that of [^{18}F]fluoro-dopamine at about 100 minutes. Very little 0-methylated metabolite was detected at any time. At any one time [^{18}F]dopamine and [^{18}F]HVA together accounted for more than 80% of the striatal ^{18}F activity. Because HVA cannot be made from dopa, without the dopa first being decarboxylated, it is clear that the combined amounts of [^{18}F]dopamine and [^{18}F]HVA must reflect the activity of aromatic acid decarboxylase. The relation between striatal ^{18}F retention after [^{18}F]fluoro-dopa administration and decarboxylase activity has not been established *in vivo* over a wide range of enzymatic activity.

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However, in a small series of experiments in which the nigrostriatal pathway of monkeys was damaged by the neurotoxin MPTP there appeared to be a relation between decarboxylase activity and ^{18}F content.[4] On the basis of the above arguments we have used [^{18}F]fluoro-dopa and positron emission tomography to make a systematic examination of the aromatic acid decarboxylase activity of human striatum.

Materials and Methods

[^{18}F]fluoro-6-L-dopa (specific activity = 200 mCi/mmol) was prepared by the method of Chirakal.[5] 3-5 mCi were injected i.v. as a bolus in saline and 1 hour later subjects were examined in the McMaster positron emission tomograph (spatial resolution 8 mm FWHM).[6] Tomographic sections were examined in a plane 10° above that through the orbitomeatal line. Each slice was 16 mm thick and overlapped its predecessor by 5 mm. In normal subjects it was possible to identify striatum in at least 5 adjacent slices. The slice that contained most striatum, by inspection, was chosen for analysis. Using an edge detection algorithm the boundaries of the striatum were identified. When suitably scaled these boundaries matched precisely those of the striatum as determined by CAT scan. They also tallied with those given in standard brain atlases. Having identified the striatal boundaries, the amount of radioactivity per pixel contained within these boundaries was determined. The average radioactivity per pixel in each of a sequence of rows, arranged transversely across the striata, was then calculated. This figure was corrected for non-specific retention of ^{18}F by the brain using the expression

$$\text{Specific } ^{18}\text{F retention} = \frac{\text{cps/pixel striatum-cps/pixel, non striatal brain tissue}}{\text{cps/pixel non striatal brain tissue}}$$

Non striatal brain tissue was measured in a region of brain in the white matter of the frontal lobes. The specific ^{18}F retention for each row of striatum was then plotted against the distance of that row from the rostral edge of the head of caudate.

Each data point was then normalized to the area under the above curve and replotted (Figure 1). By doing this all of the individuals studied were directly comparable.

Subjects/Patients

All of the individuals that were studied with [^{18}F]fluoro-dopa were studied with [^{18}F]deoxy-glucose (^{18}FDG) within 24th of the [^{18}F]fluoro-dopa study. The same tomographic slices were examined. Three healthy adults in their early twenties (a female and 2 males) and four healthy adults in their early sixties (a female and 3 males) were studied.

Five children, all girls, suffering from primary dystonia were also studied. Their ages ranged from 9 to 22 years. In one of the girls the dystonia was confined to the right (without a known cause or reason), in 3 it was generalized. In one it involved right arm and left leg. All of the girls underwent a therapeutic trial of L-dopa. In two this resulted in pronounced improvement (dopa-responsive dystonia) and in one some questionable benefit.

Eleven patients suffering from recently diagnosed idiopathic Parkinson's disease were also studied. None of the patients had been treated at the time of the study.

A single patient, a chemist, aged 51 who had previously been exposed to MPTP was also studied. He had no clinical features of parkinsonism but the concentration of HVA in his lumbar C.S.F. was 60% of normal while the corrected concentration of MHPG was elevated by 40%.

Results

Figure 1a shows the distribution of ^{18}F activity and hence aromatic acid decarboxylase activity through the striatum in a rostrocaudal direction, in the young adults. There is little or no rostrocaudal gradient.

Figure 1b shows that in the older adults there is a small but consistent reduction in decarboxylase activity in the caudal striatum. If, instead of plotting the mean of the left and right striatal ^{18}F activity, as was done in Figure 1, left to right differences were examined, it was found that there was no association between the handedness of the individuals and any left/right differences in ^{18}F activity (2 of the younger and 1 of the older group were left handed).

The distribution of ^{18}F activity within the striatum of the dystonic children, Figure 2, resembled that of the older control subjects. There was a definite but not marked rostrocaudal gradient with activity in the caudal putamen being lower. When the activities of each side were compared there was no association between the pattern of distribution of ^{18}F along the striatum and the severity or degree of one sidedness of the clinical features.

Figure 3 shows the distribution of decarboxylase activity along the striatum of parkinsonian patients. There is a marked loss of activity in the caudal putamen.

In the single MPTP exposed patient there was no rostrocaudal gradient for ^{18}F concentration.

No consistent abnormalities were found in the distribution of ^{18}FDG within the striatum either in the dystonic or parkinsonian patients.

Discussion

Several autopsy studies have examined the regional variation in neurotransmitter and amine metabolism in the striatum of normal individuals. Gaspar et al[7] found that in control subjects there were no regional differences in the striatal concentration of tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis within the striatum. A study by Nyberg et al[8] found no rostrocaudal gradient for dopamine concentration in the striatum. A similar study by Fahn et al[9] confirmed this result. Our *in vivo* findings are generally in keeping with these observations. However it seems that in young normal adults there is no gradient at all whereas in physically agile individuals in their sixties there is a gradual reduction in aromatic acid decarboxylase as one passes back through the striatum.

There are various claims in the literature that hand preference and laterality are related to asymmetries in striatal dopamine concentration.[10,11] Of all of the mammals, man is the one in whom hand preference is most marked, but there was nothing in the results from the 7 control subjects that we studied to suggest a relation between handedness and ^{18}F accumulation. Similarly there was no relation between local striatal dopamine concentration and the side of the body that was affected in the

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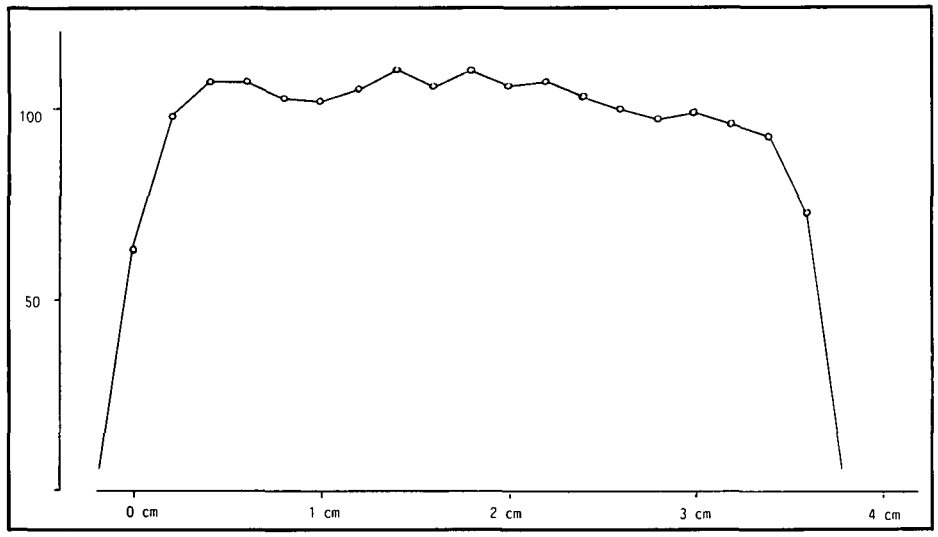

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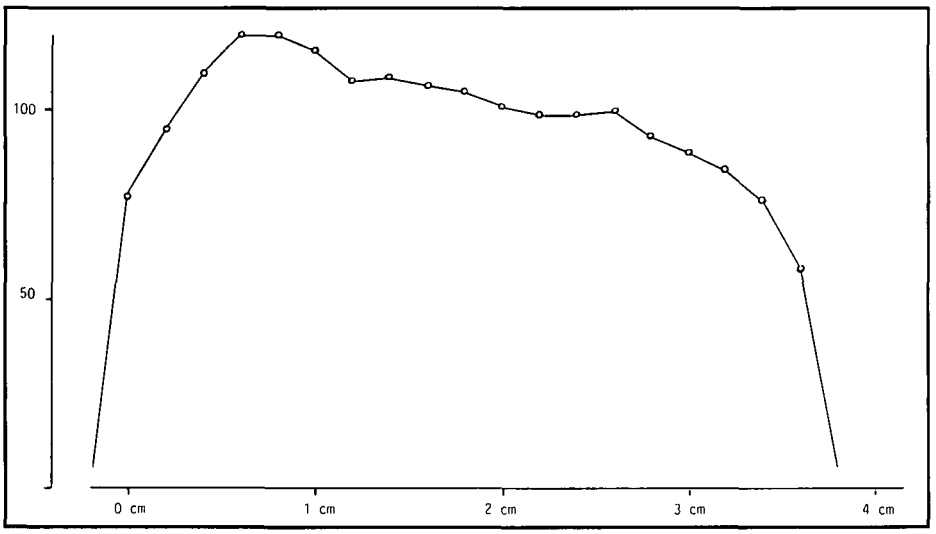

B

Figure 1A — Distribution of aromatic acid decarboxylase activity within the striatum of young control subjects. The curve represents the mean of values from the L & R striatum in 3 individuals. The ordinate in this and subsequent figures is given in arbitrary units of enzymatic activity derived as described in the text. The abscissa indicates the distance in cm from the beginning of the head of the caudate nucleus. 0-1.5 cm includes the caudate, 1.5-3.5 cm includes the putamen. Figure 1B — Distribution of aromatic acid decarboxylase activity within the striatum of adult control subjects in their early sixties (4 subjects).

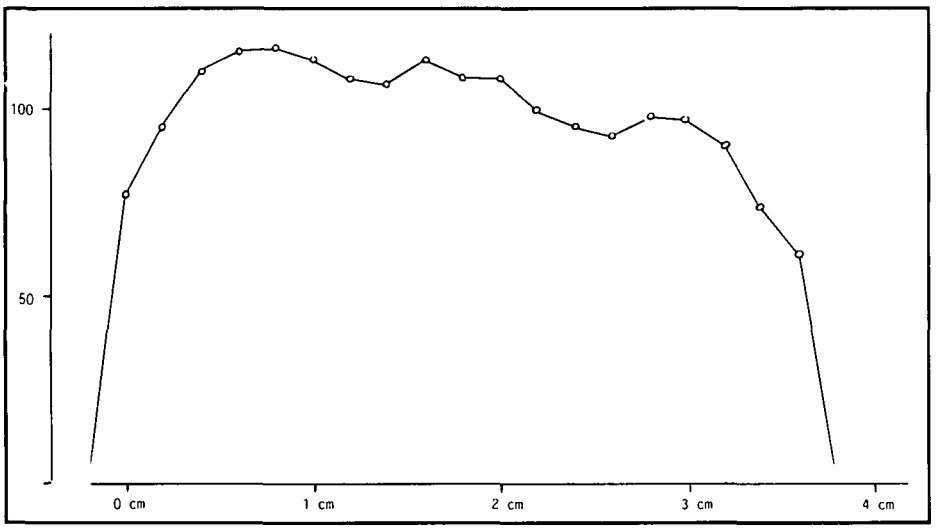

Figure 2 — Distribution of aromatic acid decarboxylase activity within the striatum of dystonic patients. The curve represents the mean of 5 patients aged from 9 to 22 years.

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children suffering from idopathic uni or bilateral dystonia. This was surprising because we have seen two individuals with unilateral secondary dystonia both of whom had a reduction of ^{18}F dopamine and ^{18}FDG accumulation in the contralateral putamen at a site that was abnormal on CAT scan. Hornykiewicz et al[12] described reductions in dopamine in one of two patients with dystonia musculorum deformans whom he studied at autopsy. The reduction of dopamine was most marked in the putamen of that patient. In the other patient there was no reduction in striatal dopamine concentration. In the five dystonic girls that we studied there was only a small rostrocaudal gradient for aromatic acid decarboxylase activity, it resembled that seen in the sixty year olds. Changes did not seem to correlate at all with benefit from L-dopa suggesting that "dopa-responsive dystonia" is not a disorder of aromatic acid decarboxylase activity.

Leenders[13] has also used [^{18}F]fluoro-dopa and PET to study adults suffering from idiopathic dystonia. He found a slight reduction in overall ^{18}F accumulation in the striatum but was unable to comment on the distribution of ^{18}F within the striatum. In essence his results are very similar to ours and suggest that alterations in total or regional striatal decarboxylase activity are not required for dystonia to be manifest.

All of the parkinsonian patients that we studied showed a marked rostrocaudal gradient for decarboxylase activity along the putamen. The caudal part was most severely affected. This gradient was steeper than that described by Nyberg[8] but it can probably be explained by the fact that our patients were not severely affected and that the nigral cells supplying the rostral putamen had not yet perished. There seems little doubt that idiopathic Parkinson's disease is characterized by a preferential loss of decarboxylase activity in the caudal putamen. The reason for this selectivity is not known. The situation seems to be different, at least in the single MPTP exposed subject that we studied. There is little doubt that this man had been damaged by the neurotoxin. He showed the characteristic reduction in HVA and elevation in MHPG that Burns (personal communication) reported after MPTP exposure yet the distribution of decarboxylase activity differs markedly from that seen even in early idiopathic Parkinson's. If this finding can be corroborated in other MPTP exposed patients it would suggest that, if Parkinson's is caused by a neurotoxin, there may be other factors that localize the effects of this toxin differently from those of MPTP.

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