Biochemical Pharmacology, Vol. 26, pp. 1271–1273. Pergamon Press, 1977. Printed in Great Britain.

COMMENTARY

MEASURING THE FORMATION OF BIOGENIC AMINES UTILIZING $^{18}O_2$

CORRADO L. GALLI, JOHN W. COMMISSIONG and NORTON H. NEFF

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeth Hospital, Washington, DC 20032, U.S.A.

The biosynthesis of the catecholamines and serotonin involves the utilization of O_2 during the enzymatic hydroxylation of their respective precursor amino acids. Therefore, $^{18}O_2$ can be substituted for $^{16}O_2$ in the inspired air of animals resulting in the labeling *in vivo* of the biogenic amines and their respective metabolites. A gas chromatograph is then used to separate the various amines and metabolites and a mass spectrometer detector distinguishes compounds containing ^{16}O from those that contain ^{18}O.

In 1973, Sedvall *et al.* [1, 2] reported that ^{18}O was incorporated into position 3 of the aromatic ring of 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA) when rats were exposed to an atmosphere of $^{18}O_2$. These studies were confirmed and expanded when it was reported that the relative abundance of ^{18}O found in dopamine from different nuclei of the same rat brain varied by about 4-fold [3]. Presumably the differences were a reflection of the rate of formation of dopamine in the nuclei. Theoretically, the measurement of ^{18}O incorporation into dopamine and its metabolites as found in blood, urine and spinal fluid would be a practical method for measuring the rate of dopamine formation in man [1–3]. There would be no need to give pharmacological agents, radioactive precursors or radioactive amines. Equipment already exists for administering gases to man, and $^{18}O_2$ is now available at a relatively reasonable cost.

Our studies have revealed, however, that the incorporation of $^{18}O_2$ into dopamine as a method for measuring its turnover is more complicated than originally anticipated [4]. We did find that the presence of [^{18}O]tyrosine in plasma after exposing animals to $^{18}O_2$ is an accurate indicator for evaluating phenylalanine hydroxylase activity *in vivo* which might serve as a diagnostic aid for identifying carriers of phenylketonuria.

Our initial studies of the incroporation of ^{18}O into dopamine were based on the observation of Mayevsky *et al.* [2] that there was no detectable incorporation of ^{18}O into tyrosine when rats were exposed to $^{18}O_2$. Consequently, [^{18}O]dopamine had to be formed during the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase and the ^{18}O atom would be at the third position rather than the fourth position of the aromatic ring. It is important to call to mind that the gas chromatography–mass spectrometry (GC–MS) procedures that are currently used to study catechol compounds cannot identify the position of the ^{18}O atom on the aromatic ring. According to the above model then, subjects could be exposed to $^{18}O_2$ for a finite period, the $^{18}O_2$ removed and the relative abundance of ^{16}O-containing catechols to ^{18}O-containing catechols could be followed with time and a kinetic model developed to estimate the rate of formation of the catecholamines. $^{18}O_2$ is rapidly exchanged with $^{16}O_2$ in the atmosphere when rats are removed from an $^{18}O_2$ exposure chamber, and the small store of ^{18}O-labelled DOPA would be quickly utilized. Thus, there would be little concern that ^{18}O-containing catecholamines would be generated for a long period after rats were removed from the chamber.

Pilot studies with $^{18}O_2$ revealed that the decline of [^{18}O]dopamine was different from our previous results using radioactive or pharmacological techniques [4]. The rates were slower than anticipated.

Table 1. Presence of [^{18}O]dopamine and metabolites in rat striatum after exposure to $^{18}O_2$ for 60 min

	Concentrations of catechol compounds containing ^{16}O and ^{18}O (nmoles/g ± S.E.M. N = 5–12)			
	Exposed to room air	Exposed to $^{18}O_2$-containing air		
	$2^{16}O$	$2^{16}O$	$^{16}O + {}^{18}O$	$2^{18}O$
Dopamine	34 ± 4	26 ± 1	6.6 ± 0.4	0.75 ± 0.01
DOPAC	4.0 ± 0.4	4.1 ± 0.3	0.75 ± 0.05	†
HVA	4.6 ± 0.5	4.3 ± 0.4	0.41 ± 0.01	†

† Rats were exposed to room air or $^{18}O_2$-containing air in a closed chamber for 60 min as described previously [1, 4]. Catechol compounds were assayed by GC–MS [4]. Each compound contains 2 atoms of O on the aromatic ring. The values indicate the concentrations of catechols containing ^{16}O, ^{18}O or ^{16}O plus ^{18}O.

† Compound was not assayed.

Table 2. Concentration of phenylalanine, tyrosine, dopamine and their ^{18}O analogues in plasma and striatum of rats after exposure to $^{18}O_2$ alone or after treatment with PCPA*

Treatment	Phenylalanine (nmoles/ml (g) ± S.E.M.)		Tyrosine (nmoles/nl (g) ± S.E.M.)		[^{18}O]tyrosine (nmoles/ml (g) ± S.E.M.)		Dopamine (nmoles/g ± S.E.M.)	[^{18}O]dopamine† (nmoles/g ± S.E.M.)
	Plasma	Striatum	Plasma	Striatum	Plasma	Striatum	Striatum	Striatum
$^{16}O_2$	73 ± 3 (13)	129 ± 6 (13)	72 ± 4 (8)	93 ± 3 (24)			33 ± 4	
$^{18}O_2$	72 ± 3 (10)	120 ± 5 (10)	63 ± 5 (8)	100 ± 7 (12)	3.1 ± 0.3 (8)	3.0 ± 0.3 (12)	26 ± 1	6.6 ± 0.4
$^{18}O_2$ + PCPA	398 ± 81‡ (14)	220 ± 19‡ (4)	127 ± 2‡ (5)	71 ± 6§ (9)	1.3 ± 0.2§ (3)	0.51 ± 0.04‡ (4)	27 ± 1	2.6 ± 0.1‡

* Rats were exposed to $^{16}O_2$- or $^{18}O_2$-containing air in a closed system for 60 min [1, 4]. Some of the rats were treated with PCPA, 300 mg/kg, i.p., 24 hr prior to exposure to $^{18}O_2$. The number of experiments is given in parentheses.

† The GC–MS was arranged to monitor dopamine containing a single atom of ^{18}O. The position of the ^{18}O atom on the aromatic ring cannot be distinguished with the presently available procedures.

‡ $P < 0.001$ when compared with $^{18}O_2$-treated animals. There was no significant difference between phenylalanine and tyrosine in rats exposed to $^{16}O_2$ or $^{18}O_2$.

§ $P < 0.01$ when compared with $^{18}O_2$-treated animals.

Table 3. Relative abundance of [^{18}O]serotonin in rat striatum after exposure to $^{18}O_2$*

Treatment	Serotonin Conc. (nmoles/g ± S.E.M.; N = 4) [^{16}O]serotonin (A)	[^{18}O]serotonin (B)	B/A
$^{16}O_2$	1.6 ± 0.3		
$^{18}O_2$	1.1 ± 0.2	0.31 ± 0.04	0.28

* Rats were exposed to $^{18}O_2$ or room air ($^{16}O_2$) for 60 min before they were killed.

A slower decline of [^{18}O]dopamine could have been the consequence of [^{18}O]tyrosine being converted to [^{18}O]DOPA and then to [^{18}O]dopamine long after the animals were removed from the $^{18}O_2$ atmosphere. The ^{18}O atom in this situation would be on the fourth position and ^{16}O on the third position of the aromatic ring. In support of this notion, dopamine containing two atoms of ^{18}O is found in rat brain after 60 min of exposure to $^{18}O_2$ (Table 1). This could only occur if ^{18}O was first incorporated into phenylalanine by phenylalanine hydroxylase and then a second atom of ^{18}O added when tyrosine was converted to DOPA by tyrosine hydroxylase. 3,4-Dihydroxyphenylacetic acid (DOPAC) and HVA containing a single atom of ^{18}O were also present in rat brain, but as already mentioned the GC–MS cannot distinguish whether ^{18}O is at position 3 or 4 of the aromatic ring. A report has recently appeared indicating that HVA containing two atoms of ^{18}O is found in the cerebrospinal fluid (CSF) of the baboon after exposure to $^{18}O_2$ [5]. The basic problem which restricts the usefulness of this technique for estimating catecholamine turnover is in interpreting the meaning of the changes of [^{18}O]-dopamine and its metabolites after exposure to $^{18}O_2$.

To aid in the evaluation of the $^{18}O_2$ studies, we developed a sensitive GC–MS procedure for assaying phenylalanine and tyrosine in tissues [4]. As shown in Table 2, substantial quantities of [^{18}O]tyrosine are indeed formed *in vivo* during 1 hr of exposure to $^{18}O_2$. To test whether the incorporation of $^{18}O_2$ was a result of the enzymatic hydroxylation of phenylalanine by phenylalanine hydroxylase, rats were treated with the phenylalanine hydroxylase inhibitor, *p*-chlorophenylalanine (PCPA), 24 hr prior to exposure to $^{18}O_2$. As a result of this treatment, there was a significant fall of the levels of [^{18}O]tyrosine in plasma and striatum. Moreover, there was also a significant decrease in the levels of [^{18}O]dopamine in striatum. Apparently drugs that alter phenylalanine hydroxylase activity could potentially modify the kinetics of decline of [^{18}O]dopamine and yet not actually change dopamine turnover. Of greater importance, however, is the potential usefulness of $^{18}O_2$ for evaluating the activity of phenylalanine hydroxylase *in vivo* in human subjects from samples of plasma after breathing $^{18}O_2$. Homozygotes for phenylketonuria are readily detected, but heterozygotes, because of attenuated enzyme activity, cannot usually be identified by simple means [6]. The $^{18}O_2$ procedure could be performed under steady-state conditions and, thus, eliminate the need to use radioactive phenylalanine or to give large amounts of phenylalanine, which upsets the normal balance of amino acids in the body, to follow the formation of tyrosine as it has been done in the past to identify heterozygotes for phenylketonuria [6].

In more recent studies, we found that ^{18}O was incorporated into brain serotonin in rats exposed to an atmosphere of $^{18}O_2$ (Table 3). Studies of the serotonin system in man should be less complicated because there is only one mono-oxygenase involved, tryptophan hydroxylase, and only one position that could be labeled, the fifth position. The relative abundance of [^{18}O]serotonin to [^{16}O]serotonin in rat brain was high after exposure to $^{18}O_2$, which is in keeping with previous reports that the rate of serotonin formation is rapid in rat brain [7].

In summary, the use of $^{18}O_2$ to explore biochemical reactions *in vivo* and to understand the mechanism of action of drugs is just beginning. Our animal studies and those of others demonstrate that human studies are feasible. There are, however, pitfalls that should be carefully considered. The potential usefulness of $^{18}O_2$ to detect a metabolic deficiency in humans is limited only by the imagination of the investigator.

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