# OXYGEN-18 IN MEASUREMENT OF DOPAMINE TURNOVER IN RAT BRAIN

GÖRAN SEDVALL AVRAHAM MAYEVSKY, DAVID SAMUEL
and CLAES-GÖRAN FRI
Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden, and the
Isotope Department, The Weizmann Institute of Science, Rehovot, Israel

INTEREST for dopamine (DA) in brain function was stimulated by the discovery that levels of this compound in brain of parkinsonian patients were markedly reduced (HORNYKIEWICZ, 1966). The demonstration that *amphetamine* (COSTA and GROPPETTI, 1970) and antipsychotic drugs markedly affect brain DA metabolism (NYBÄCK and SEDVALL, 1968) indicated a role for this transmitter substance also in psychotic states like *schizophrenia* (KLAWANS *et al.*, 1972).

For studies on brain DA metabolism in man, determination of base line levels or *probenecid* induced elevations of homovanillic acid (HVA), the major DA metabolite, in lumbar cerebrospinal fluid (CSF) seem to be the best procedures elaborated so far (PAPESCHI, 1972; SJÖSTRÖM, 1973). However, since mechanisms for HVA transport from brain to lumbar cerebrospinal fluid are not readily controlled and probenecid in tolerable doses only gives a sub-maximal blockade of HVA transport from CSF, the search for alternative methods is necessary. In animals the use of catecholamine synthesis inhibitors or precursors labelled with radioactive isotopes seem to be the most valid procedures developed so far for quantitative determination of brain catecholamine turnover (COSTA, 1972).

The development of mass spectrometric methods for determination of catecholamines and their metabolites (ÄNGGÅRD and SEDVALL, 1969; KOSLOW, CATTABENI and COSTA, 1972, FRI *et al.*, 1973, in preparation) has recently made possible the use of stable isotopes to label brain catecholamines *in vivo* for turnover determinations. Thereby the risk with radioactive isotopes can be avoided. Since catechol- and indoleamines are formed in rate limiting reactions by hydroxylases using molecular oxygen, the possibility to label brain monoamines *in vivo* with stable oxygen isotopes is of considerable interest. Molecular oxygen can easily be administered by inhalation and by a single exposure it is possible to label a number of compounds of biological interest.

In a recent series of experiments we could demonstrate the use of *stable oxygen isotopes* for labelling of HVA in rat brain. Following *in vivo* exposure of rats to atmospheres highly enriched with oxygen-18, mass spectrometric evidence was obtained for the incorporation of at least one oxygen isotope in brain HVA (SEDVALL *et al.*, 1973, MAYEVSKY *et al.*, 1973). Figure 1 demonstrates the high mass range in the mass spectra of the methyl ester heptafluorobutyryl derivative of authentic HVA and apparent HVA from brain extracts. HVA has the molecular ion at m/e 392 which is also the base peak. An abundant fragment is also present at m/e 333. An almost identical spectrum was obtained from brain extracts of animals exposed to an $^{16}O_2$ containing atmosphere. On the other hand, in animals exposed to $^{18}O_2$ gas, abundant

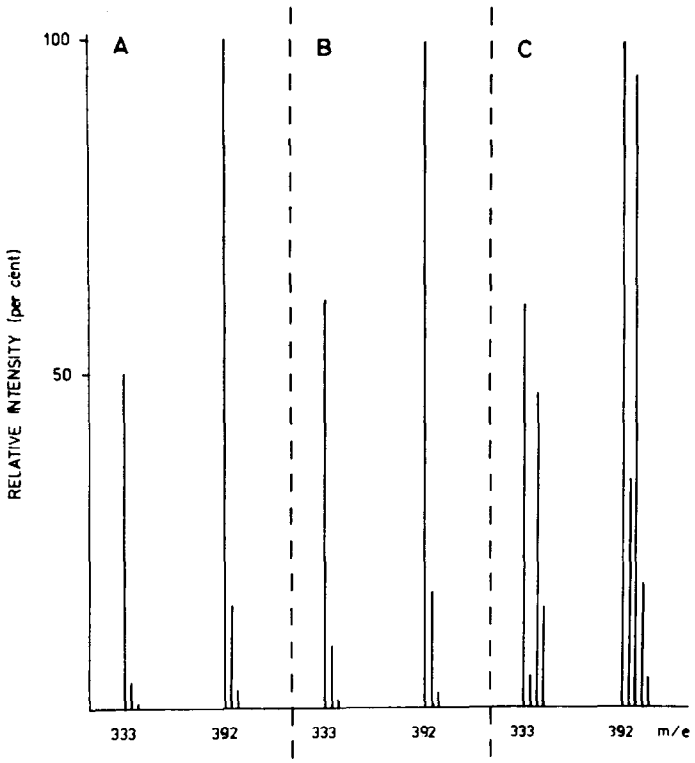


FIG. 1.—High mass range of HVA in the form of Me-HFB derivatives. A represents authentic HVA. B and C are obtained from brain of rats exposed to $^{16}O_2$ and $^{18}O_2$ respectively. Spectra were obtained by subtracting background intensities at retention time 4·15 min from values recorded at 4·20 min—the retention time of the HVA derivatives on the 1% OV-17 column used.

fragments at m/e 334 and 335 were also present, demonstrating the presence of HVA containing one $^{18}O$ atom per molecule.

The atmospheric origin of the $^{18}O$ atom incorporated was proved by injecting $^{18}O$ labelled water into the animals which was not followed by incorporation of significant amounts of $^{18}O$ in brain HVA (MAYEVSKY *et al.*, 1973). The usefulness of the technique for studies on brain DA turnover was demonstrated by studying the effect of chlorpromazine treatment on $^{18}O$, incorporation in brain HVA. Chlorpromazine treatment was followed by an approximately three-fold increase in the rate of formation of $^{18}O$ labelled HVA in the rat brain (SEDVALL *et al.*, 1973). For the quantitative determination of DA turnover an analysis of precursor–product relationships for the incorporation of $^{18}O$ in precursor amino acids, DA and HVA is required. For that purpose mass fragmentometric methods for the determination of phenylalanine, tyrosine, DA and HVA in the same rat brain sample have been developed (FRI *et al.*, 1973, in preparation). In the present communication mass fragmentometric evidence for the incorporation of $^{18}O$ in DA of rat brain during *in vivo* exposure to $^{18}O$ containing atmospheres is presented.

## METHODS

Male Sprague–Dawley rats weighing about 60 g were introduced into a system (MAYEVSKY *et al.*, 1973), which contained normal air at the beginning of the

experiment. As the system was closed and oxygen was consumed by the animals, $^{18}O_2$ or $^{16}O_2$ gas containing about 95% $^{18}O$ or 100% $^{16}O$ was introduced by a pressure regulated valve. Humidity and carbon dioxide formed within the system were removed by circulating the atmosphere over silica gel and a $CO_2$ trap. Immediately after the end of exposure, which lasted 3 hr, the animals were removed from the system, decapitated and the corpora striata were dissected out and homogenised in 0·1 M formic acid containing 0·5 $\mu$M ascorbic acid. Aliquots of the extracts were analysed for DA and HVA by mass fragmentographic procedures described by KOSLOW, CATTABENI and COSTA (1972) and FRI *et al.*, (1973). For dopamine determination *α-methyldopamine* (α-MDA) was used as internal standard, for HVA determination a deuterated HVA molecule was used. A schematical outline of the chemical procedures used is depicted in Fig. 2. For further details see MAYEVSKY

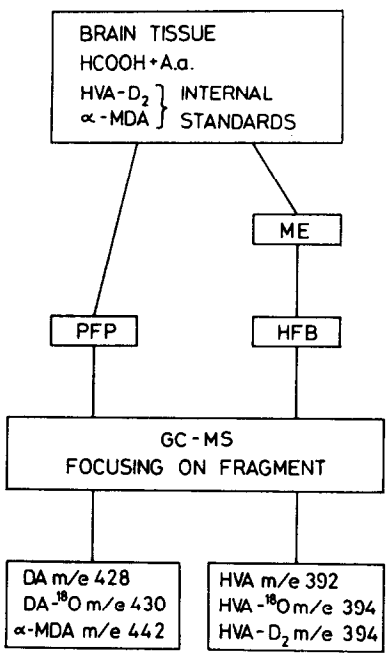


FIG. 2.—Flow sheet for extraction, derivatisation and mass fragmentometric analysis of DA and HVA in rat brain tissue.

*et al.* (1973). Recordings were made using an LKB 9000 gas chromatograph-mass spectrometer.

## RESULTS

Mass spectra of pentafluoropropionic (PFP) acid derivates of DA and α-MDA were shown by KOSLOW *et al.* (1972) to exhibit major fragments at m/e values 428 and 442 respectively. The latter authors also reported the mass fragmentometric identification of DA in rat striatum. Figure 3 demonstrates a mass fragmentogram from authentic DA and DA extracted from striata of rats exposed for 3 hr to $^{16}O$ and $^{18}O$ containing atmospheres respectively. The figure illustrates how in control rats significant intensities are obtained at m/e 428 but not 430 as in authentic DA. In $^{18}O$ exposed animals however, there is a relative diminution of the intensities at m/e 428,

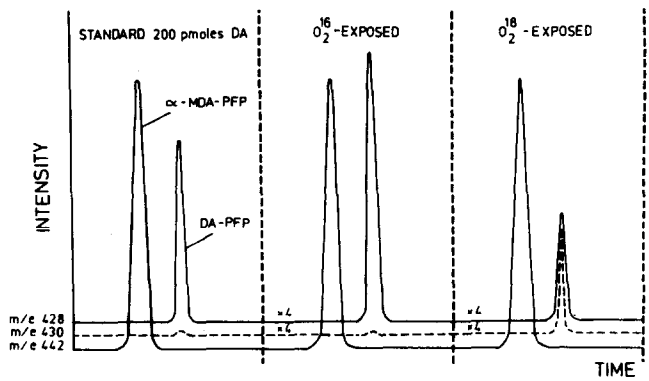


FIG. 3.—Mass fragmentograms of pentafluoropropionyl derivates of DA extracted from brain of (B) $^{16}O_2$ and (C) $^{18}O_2$ exposed rats. The curve for m/e 442 represents the derivative of the internal standard α-MDA. M/e 428 represents authentic DA, whereas 430 represents DA labelled with one $^{18}O$ atom.

whereas significant intensities are obtained also at m/e 430. By mass fragmentography and the use of α-MDA and HVA-$D_2$ as internal standards the absolute amounts of $^{16}O$ and $^{18}O$ labelled DA and HVA were determined in brains from $^{16}O$ and $^{18}O$ exposed animals. It is evident from Table 1 that following exposure to an $^{18}O$ con-

TABLE 1. OXYGEN ISOTOPIC LEVELS (pmole/striata) OF DA AND HVA IN RAT BRAIN AFTER EXPOSURE TO $^{16}O_2$ OR $^{18}O_2$

| Exposure | DA | DA-$^{18}O$ | HVA | HVA-$^{18}O$ | % $^{18}O$ in air |
|---|---|---|---|---|---|
| $^{16}O_2$ | 2860 ± 310 | not detect. | 422 ± 31 | not detect. | 0·2 |
| $^{18}O_2$ | 1130 ± 400 | 103 ± 130 | 222 ± 16 | 186 ± 18 | 81·5 |

Data represent mean ± S.E. from 5 to 6 rats.

taining atmosphere for 3 hr approximately 50% of the amount of DA as well as HVA are labelled with $^{18}O$. The mass spectrometer was also focussed on m/e 432, which should represent DA labelled with two $^{18}O$ atoms. Also here intensities significantly above back-ground were obtained, but they were too small to allow exact quantification.

## DISCUSSION

We have previously demonstrated the incorporation of one $^{18}O$ atom into HVA of rat brain following *in vivo* exposure to $^{18}O$ containing atmospheres. The present experiments give strong evidence for the labelling also of DA with $^{18}O$. This is a direct *in vivo* confirmation of previous *in vitro* data demonstrating that hydroxylation of tyrosine to DOPA involves the incorporation of molecular oxygen (NAGATSU, LEVITT and UDENFRIEND 1964, DALY *et al.*, 1968). Since mass fragmentometric methods for the determination of phenylalanine, tyrosine, DA and HVA in single rat striata were recently developed (FRI *et al.*, 1973, in preparation), the prerequisite for determination of precursor-product relationships for $^{18}O$ incorporation in the DA pathway are now available.

Inhalation of $^{18}O$ containing atmospheres for several months in rats was not followed by any signs of toxicity (SAMUEL, 1973, in preparation). The availability of

techniques for the mass fragmentometric determination of HVA levels in lumbar CSF, serum and urine of man makes it possible therefore to initiate studies with $^{18}O$ for determination of brain DA turnover also in humans.

For clinical studies $^{18}O$ gas could easily be administered by a short term inhalation from a closed system. Labelling of brain monoamines by such a procedure and subsequent determination of changes in specific activity of amine metabolites in CSF should allow calculation of brain DA turnover rates. By measuring specific activities in serum or urine following blockade of peripheral HVA synthesis at optimal intervals following $^{18}O$ exposure, selective determination of brain DA synthesis could also be obtained. Future studies have to elucidate the practicability of such methods which might be of value for studies on brain DA synthesis during neuropsychiatric disease states. Such procedures might also be of value to control specific drug therapies directed to alteration of brain DA metabolism. Besides for DA, the described technique may be preferable also to study the metabolism of *noradrenaline* and *serotonin* in the central nervous system, since the latter amines too are formed by hydroxylases using molecular oxygen.

*Acknowledgements*—The present study was supported by the Swedish Medical Research Council (40X-3560) and the Weizmann Institute of Science, Israel. The skilful technical assistance of Mrs. Varda Greenberger is gratefully acknowledged.

## REFERENCES

COSTA E. (1972) *Adv. Biochem. Psychopharmacol.* **4,** 171–193.
COSTA E. and GROPPETTI A. (1970) In: *International Symposium on Amphetamines and Related Compounds.* (COSTA E. and GARATTINI S., Eds.) pp. 231–255. Raven Press, New York.
DALY J., LEVITT M., GUROFF G. and UDENFRIEND S. (1968) *Archs. Biochem. Biophys.* **126,** 593–598.
HORNYKIEWICZ O. (1966) *Pharmacol. Rev.* **18,** 925–964.
KLAWANS H. L., GOETZ B. A. and WESTHEIMER R. (1972) *Dis. Nerv. Syst.* **33,** 711–719.
KOSLOW S. H., CATTABENI F. and COSTA E. (1972) *Science* **176,** 177–180.
MAYEVSKY A., SJÖQUIST B., FRI C. -G., SAMUEL D. and SEDVALL G. (1973) *Biochem. Biophys. Res. Comm.* **51,** 746–756.
NAGATSU T., LEVITT M. and UDENFRIEND S. (1964) *J. Biol. Chem.* **239,** 2910–2917.
NYBÄCK H. and SEDVALL G. (1968) *J. Pharmacol. Exp. Ther.* **162,** 294–301.
PAPESCHI R. (1972) *Psychiat. Neurol. Neurochir.* **75,** 13–48.
SEDVALL G., MAYEVSKY A., FRI C. -G., SJÖQUIST B. and SAMUEL D. (1973) *Adv. Biochem. Psychopharmacol.* **7,** in press.
SJÖSTRÖM R. (1973) *Acta Universitatis Upsaliensis* **154,** 5–39.
ÄNGGÅRD E. and SEDVALL G. (1969) *Anal. Chem.* **41,** 1250–1256.