An On-Line Synthesis of $[^{15}O]N_2O$: New Blood-Flow Tracer for PET Imaging

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This paper describes a recently developed on-line synthesis of a new blood-flow tracer, O-15-labeled nitrous oxide. The tracer was produced by catalytic oxidation of anhydrous ammonia in a gas mixture containing O-15-labeled molecular oxygen. (The oxygen-15 was produced by a $^{14}N(d,n)^{15}O$ reaction.) Anhydrous ammonia was mixed with the gas containing $[^{15}O]O_2$, and after preheating to about 200°C was carried through an oven containing a Pt catalyst kept at about 310°C. Labeled gas was purified in H_3PO_4 and KOH traps. O-15-labeled nitrous oxide was identified by gas radiochromatography and by various chemical reactions. Radiochemical purity of the O-15-labeled nitrous oxide exceeded 98%, radiochemical yield corrected for radioactioactive decay was 15–20%, and specific activity at the end of synthesis was about 50 mCi/mmole.

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In the past few years computer-assisted emission tomography has gained considerable importance in the quantitative in vivo measurement of regional cerebral blood flow (rCBF) in humans. The methods used are single-photon computer-assisted tomography (*1,2*) and positron emission tomography (*3–6*) (PET).

Positron-emitting radioactive tracers can be divided into two groups: inert gases (Kr-77, Ne-19, Xe-123) and freely diffusible tracers ($[^{15}O]H_2O$, $[^{15}O]CO_2$, $[^{11}C]$-iodoantipyrine, $[^{11}C]$methane, $[^{13}N]$nitrous oxide, $[^{18}F]$fluoroalkenes). From this long list only a few tracers have been used for quantitative rCBF measurements in vivo in humans: Kr-77 (*3*), $[^{15}O]H_2O$ (*6*), $[^{15}O]CO_2$ (*5*) and $[^{18}F]CH_3F$ (*7*). Most of these tracers, however, have certain shortcomings. For example, their extraction in the brain is not a linear function of the blood flow (e.g. $[^{15}O]H_2O$ (*8*)) and solubility in the blood for some of them is low (Kr-77 solubility coefficient = 0.085). Furthermore, equilibrium concentration in the arterial blood is greatly influenced by delivery of the tracer ($[^{15}O]CO_2$) (*9*) and by any change in physiological conditions during measurement. For lack of anything better, nevertheless, these methods have been used for measurement of regional blood flow.

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Although use of C-11-labeled 4-iodoantipyrine (IAP) as an rCBF tracer with PET has been suggested (*10*), it has not yet been used. The only tracers used in this way are Kr-77 and O-15-water (with very fast PET scanning.)

The supply of Kr-77 radiokrypton is limited to centers with access to a large cyclotron (*11*), which is rare in hospitals or PET centers. The need for another good rCBF tracer has been obvious for some time. Analysis of the prospective candidates ($N_2{}^{15}O$, $[^{13}N]N_2O$, $[^{18}F]CF_3I$ and some others) led us to nitrous oxide, the original tracer Kety (*12*) used for noninvasive measurement of rCBF. The great advantage of nitrous oxide as an rCBF tracer is that its blood/tissue partition coefficient (*13,14*) is close to one (1.07). There are two possibilities for nitrous oxide labeling: either by O-15($T_{1/2}$ = 2 min) or by N-13($T_{1/2}$ = 10 min). The former is the more attractive option because the radiation dose to the patient is one-fifth that of the latter (unpublished data). Moreover, use of N-13-labeled nitrous oxide limits one to a dynamic mode of imaging; this produces scans of lower spatial resolution because there are fewer coincidences collected in each slice. In addition, a large radiation dose is received by the radiochemist because of N-13-nitrous oxide's lengthy preparation time (*15*).

These considerations prompted us to develop on-line synthesis of O-15-labeled nitrous oxide. Our synthesis is based on the oxidation of anhydrous ammonia over a

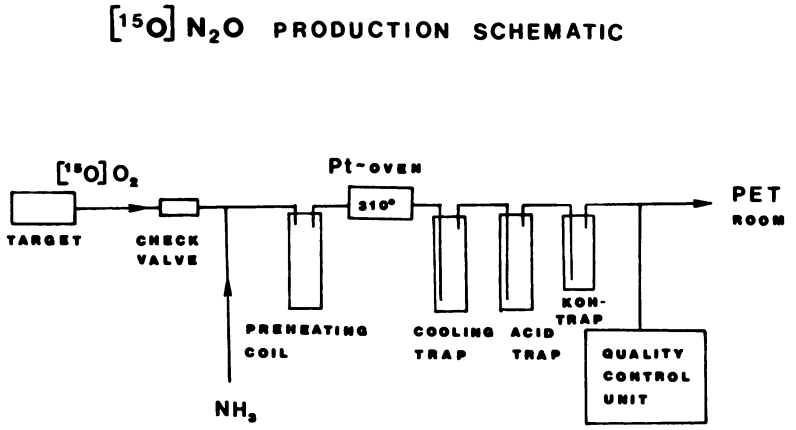

FIG. 1. Schematic diagram of $[^{15}O]N_2O$ production apparatus

platinum catalyst at a relatively low temperature of ~310°C.

EXPERIMENTAL METHODS

We have investigated several possible reactions for on-line production of nitrous oxide [O-15]. Reaction between nitrogen and oxygen has been induced by a high frequency electrical discharge (*16*), microwaves (*17*), or by a high ionization field (*18–20*). (A rather high ionization field is present in the target box during cyclotron irradiation.) Chemical reaction between nitrogen and atomic oxygen, the latter produced mainly by a $^{14}N(d,n)^{15}O$ reaction but also by dissociation from molecular oxygen under an irradiation field, will yield nitrous oxide and other nitrogen oxides (*19,20*). These are produced by a complex mechanism that has been studied extensively under different irradiation conditions (*17,19,21*). The reaction mechanism is beyond the scope of this paper. We shall accept evidence in the literature that $[^{15}O]N_2O$ is produced in the irradiated nitrogen (*19,20*).

Production of N_2O. We have investigated the amount of nitrous oxide produced by irradiation of nitrogen gas of various degrees of purity*. (Nitrogen always contains small amounts of oxygen.) Oxygen-15 produced by a $^{14}N(d,n)^{15}O$ reaction is in a high-excitation atomic state and is chemically very reactive. A high radiation field present in the target gas also produces atomic nitrogen from the molecular nitrogen used as target material (*22*). The latter, also very reactive, can react with oxygen, producing nitrogen oxide (*17–21,22*). Our experiments have also shown that it is possible to produce nitrous oxide directly in the target under our irradiation conditions (50 μA deuteron current): we have produced 1–5% of nitrous oxide(O-15) in this way by irradiating prepurified nitrogen and removing other nitrogen oxides by KOH (*23*) and phosphoric acid (*24*) traps. We used target gases having 3 ppm to 2% of oxygen in nitrogen. Target gases were under 4 atm pressure and the target was swept with these gases at a rate of 70 ml/min. The O-15-radioactivity, after passing through the traps described above, was present in the form of molecular oxygen and nitrous oxide; we were not able to remove oxygen without destroying nitrous oxide. The presence of nitrous oxide [O-15] in irradiated gas was also reported earlier (*20*).

The amount of nitrous oxide could be increased to almost 10% by passing gas after irradiation through an ozonizer† and purifying it as mentioned earlier. However, we found it extremely difficult to remove the oxygen; it also carries an O-15-label and would distort physiological measurements carried out with nitrous oxide. We have investigated the so-called oxitrap‡, pyrogallol, and Cu-powder traps. The first two removed oxygen only partially, reducing it to about 30%. The Cu-powder trap, operating between room temperature and 200°C, removed the oxygen but also reduced nitrous oxide to elementary nitrogen, so that the label was lost and remained in the oven bound to copper. We realized that the only method that might prove satisfactory was catalytic oxidation of ammonia. The net reaction of ammonia oxidation can be described as:

$$2NH_3 + 2O_2 \xrightarrow[\text{temperature}]{\text{catalyst}} N_2O + 3H_2O$$

Oxygen-15 was produced by the $^{14}N(d,n)^{15}O$ reaction in our medical cyclotron. The nominal deuteron energy on the target gas is about 6.2 MeV. The nitrogen, containing 4% of oxygen, was irradiated in an aluminum target box with a 20-μm aluminium window. Any impurities such as $[^{15}O]CO_2$ and $[^{15}O]NO_2$ were removed by a soda-lime trap from the oxygen mainstream (*20*) before the gas was mixed with anhydrous ammonia. The radiochemical purity of $[^{15}O]O_2$ was assayed by gas radiochromatography at better than 99%. There was a small trace (<1%) of $[^{15}O]CO_2$.

After the target was mixed with a closely controlled

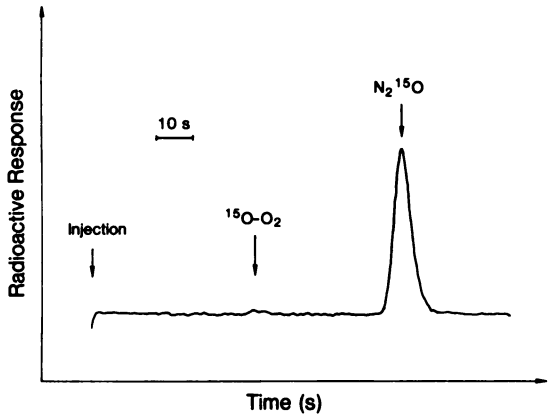

FIG. 2. Gas radiochromatogram of O-15-labeled nitrous oxide; conditions are given in text.

flow of anhydrous ammonia, it was carried through a preheating loop kept at about 200°C (Fig. 1). The system consists of several stages:

(a) production of O-15 by irradiation of a mixture of 4% O_2 in highly purified nitrogen at a pressure of 4 absolute atmospheres;
(b) mixing of $[^{15}O]O_2$ with anhydrous ammonia at a molar ratio of about 2:1 (O_2/NH_3);
(c) preheating of the coil;
(d) oxidation of ammonia in an oven with Pt catalyst;
(e) separation of unreacted ammonia and water (produced by oxidation of ammonia) in the H_3PO_4 trap; and
(f) removal of traces of acid, if any, from the gases in the KOH trap.

The purification of nitrous oxides with these two traps (KOH and H_3PO_4) has been described earlier (*23,24*).

RESULTS AND DISCUSSION

Preheated gases enter a 310°C oven containing a Pt-wire catalyst in a quartz tube. The amount of catalyst is critical for complete utilization of oxygen. We used 5 g of Pt wire, washed in dilute hydrochloric acid and deionized water, and packed into a quartz tube of 8 mm i.d. (the catalyst is reusable and good for a long time). After leaving the oven, gases are carried through a concentrated phosphoric acid trap to remove any non-reacting ammonia and water from the reaction gases. At that point nitrous oxide is radiochemically pure (>98%) and is delivered with the nitrogen (target gas) mixture to the positron emission tomography area. The chemical purity was assayed by gas chromatography using the same conditions as described for nitrous oxide. We were not able to detect any ammonia or nitric oxide in the final gas. We estimated the upper limit for the presence of chemical impurities, excluding the nitrogen target gas, as less than a few ppm in the final labeled gas.

TABLE 1. YIELD OF $[^{15}O]N_2O$ AS A FUNCTION OF PREHEATING Pt OVEN TEMPERATURE AND FLOW

Flow ml/min	Preheating Coil temp. °C	Pt-Oven Temp. °C	Activity $[^{15}O]N_2O$ produced mCi/min
50	200	290	0.3
50	200	310	1.5
50	200	330	2.0
50	200	350	1.8
50	room	320	traces
50	room	340	traces
75	200	310	4.5
75	200	330	5.0
75	200	350	4.5
75	room	330	0.5
75	room	350	0.5
100	200	315	10
100	200	340	10
100	200	360	9

We have observed that for the first 5–10 min, the oxidation of ammonia on the newly prepared catalyst produces a mixture of nitrous oxide, nitric oxide, and water (*26–28*). The amount of nitric oxide gradually decreases until the product is pure nitrous oxide. Nitric oxide was identified by gas radiochromatography, comparing retention time for the radioactivity with an authentic sample of NO. The identity was confirmed by reduction of NO in a Cu_2O oven (*24*).

GC analysis. The nitrous oxide is tested for purity on a gas chromatograph (GC) with a 1/8″ o.d. Porapak Q (80–120 mesh) column and a thermal conductivity detector. The injector, detector, and column are maintained at 100°C. Helium at a flow of 23 ml/min was used as a carrier gas. The entire synthesis, including purification and quality control, is remotely controlled to minimize exposure of the personnel as well as to reduce possible human error during on-line production of the tracer for patient use. Figure 2 shows a gas radiochromatogram. As can be seen, almost all radioactivity is in the form of nitrous oxide, whose radiochemical purity exceeds 98%.

Effects on yield. We have investigated the effect on the yield of O-15-labeled nitrous oxide of various flow rates, the temperature of the preheating coil and oven, and the ratio between oxygen and ammonia. Our results are summarized in Table 1.

It is obvious that the gas must be preheated to achieve the maximum yield of nitrous oxide. We have not been able to measure directly the temperature of the gases coming from the preheating coil, but have clearly established the need for this step. Other investigators who have studied ammonia oxidation as a means to produce

nitric acid (*25,26*) have come to the same conclusion (*27*).

The kinetics of the ammonia oxidation have been extensively investigated and several reaction sequences (*27,28*) have been proposed. Their consideration is beyond the scope of this paper.

We also studied the influence of the Pt-oven temperature on the yield and purity of N_2O. The results (Table 1) reveal that the oven temperature is critical in the production of $[^{15}O]N_2O$: the yield increases as the temperature rises to about 300°C, and then levels off. The maximum yield is obtained in the narrow temperature range of 300–340°C; above this it decreases sharply as a result of thermal decomposition of nitrous oxide and a change in the oxidation mechanism (*27*). This effect of temperature on the yield of nitrous oxide in the oxidation of ammonia has already been observed (*28*).

In contrast to other nitrogen oxides, nitrous oxide is a relatively inert gas chemically, and its chemical identification could be derived only indirectly. In our work we have applied some tests used in the past for identification of nitrous oxide (*20,28,29*). We mention them as complementary evidence that our radioactive gas is a nitrous oxide. Yet another gas to be expected in ammonia oxidation is nitric oxide (*28*). It was shown earlier that nitric oxide reacts with O_2 giving NO_2 (*30*) and is reduced by Cu_2O at 250°C (*24*), and by a basic solution of pyrogallol (*31*). All chemical tests with radioactive gas were negative, yielding the radioactive gas with the same characteristics as that entering the traps.

Specific activity. The specific activity of O-15-labeled nitrous oxide is determined by measuring mass with a gas chromatograph and radioactivity with an radionuclide calibrator. The mass response of the detector was calibrated with a known amount of nitrous oxide; the specific activity obtained at the time of synthesis was about 50 mCi/mmole of nitrous oxide. This is sufficiently high that the physiological effects of nitrous oxide described in the literature (*32*) should not be cause for concern. In absolute terms the patient would receive less than 1% of nitrous oxide in medical air.

CONCLUSION

On the basis of chromatographic identity with an authentic sample of N_2O and chemical nonreactivity used only as complementary evidence, we conclude that the gas must indeed be O-15-labeled nitrous oxide.

The radiochemical yield, corrected for radioactive decay, is 15–20%. Improving this yield is our next challenge.

FOOTNOTES

* Matheson purity and high-purity nitrogen supplied by Matheson of Canada.

† Ozonizer—Cat. No. 6-0615, Supelco. Inc., PA.

‡ Oxitrap—Cat. No. A4001, Altech Associates.

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