PREPARATION AND PURIFICATION OF ^{77}Br-LABELLED p-BROMOSPIROPERIDOL SUITABLE FOR IN VIVO DOPAMINE RECEPTOR STUDIES*

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SUMMARY

An efficient and reliable method of preparing ^{77}Br-p-bromo spiroperidol suitable for labelling dopamine receptors *in vivo* has been developed. This synthesis involves the reaction of $^{77}Br^-$, H_2O_2, and spiroperidol in glacial acetic acid. The purity of the product was assured by the use of two column reverse phase high performance liquid chromatography. Radiochemical yields were in the range of 80-90%, while specific activities of 80-140 Ci/mmole were obtained at the end of the synthesis.

Key Words: radiobromination, ^{77}Br-p-bromospiroperidol, receptor-targeted radiopharmaceutical, dopamine receptors

INTRODUCTION

Spiroperidol (SP) (8-(p-fluorobenzoyl)propyl-1-phenyl-1,3,8-triazaspiro-[4.5]-decan-4-one) (I) is a potent dopamine (DA) antagonist which, because of its high binding affinity and specificity for DA receptors, has been an extremely useful ligand in the delineation and characterization of DA binding sites in the brain and other tissues (1). However, for the purpose of mapping DA receptors *in vivo*, a non-invasive imaging procedure involving a gamma or positron emitting SP analog would be most appropriate. This approach has led to the development of methods

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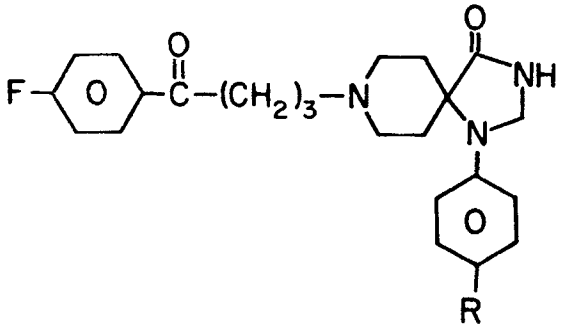
to synthesize spiroperidol labelled with short-lived positron emitters, ^{11}C ($t_{1/2}$= 20 min) and ^{18}F ($t_{1/2}$= 110 min) (2,3). ^{11}C-spiroperidol, because of its short half-life, offers the potential for doing serial studies but whether the constraints of its half-life would hinder its utility is currently being investigated (2). On the other hand, the synthesis of ^{18}F-spiroperidol has so far resulted in low radiochemical yields (3). Recently, a brominated analog, p-bromospiroperidol (BrSP) (8-(p-fluorobenzoyl)propyl-1-(p-bromo)phenyl-1,3,8-triazaspiro-[4.5]-decan-4-one) (II) was synthesized in this laboratory and was found, using in vivo and in vitro tests, to essentially retain the biologic activity of SP (4). Several presently available bromine radioisotopes, ^{75}Br, ^{76}Br, and ^{77}Br, are well-suited for external gamma detection (5), and can serve as labels for a radiobrominated BrSP.



I: R=H
II: R=Br

^{77}Br labelled BrSP has been prepared in this laboratory and the specific in vivo localization of this drug was studied in rat brain. The results have shown that the distribution of this radiotracer very closely parallels that of ^{3}H-SP (6). A further development more recently saw the successful imaging,

using a gamma camera as a single photon tomograph, of the brain of an intact cat injected i.v. with ^{77}Br-BrSP (7). Higher uptake, slower wash-out, and specific displacement with unlabeled SP was observed over a five-hour time course in areas, such as the striatum, which are known to be rich in DA receptors, compared to the cerebellum, a DA receptor-poor area. Furthermore, the striatum: cerebellum ratio of the ^{77}Br-BrSP concentrations, which is related to the specific binding of the drug, measured from the imaging results was found to closely agree with the ratio obtained when dissected brain tissues from the same cat were analyzed.

This demonstrates that the use of a gamma emitting SP analog in the non-invasive measurement of DA receptor parameters is indeed a very promising approach. Such measurements could lead to a better understanding of the changes in DA receptors which have been implicated in several CNS diseases such as Parkinson's disease, Huntington's chorea, and schizophrenia. This would hopefully impact the diagnosis and treatment of these diseases.

The following report details our efforts toward the development of a rapid, efficient, and consistent procedure to prepare ^{77}Br labelled BrSP for use in the in vivo studies of dopamine receptors.

METHODS AND MATERIALS

Spiroperidol and Analogs

Spiroperidol was synthesized using essentially the method of Janssen (8). γ-Chloro-p-flourobutyrophenone was refluxed with 1-phenyl-1,3,8-triazaspiro-[4.5]-decan-4-one, both obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, in methyl ethyl ketone for 48 hours with stirring. Also added to the reaction mixture was Na_2CO_3 and a few crystals of KI. At the end of the reaction, the mixture was cooled and water was added. SP was extracted

into CH_2Cl_2 and the crude product was purified by recrystallization from 1:1 CH_2Cl_2-CCl_4. The latter step was repeated as needed, until a single peak was obtained by reverse phase high performance liquid chromatography. This peak had previously been assigned to spiroperidol using an authentic sample obtained from Janssen Pharmaceuticals, Belgium.

Mass spectrometry, NMR analysis and melting point (190-193°C) confirmed that the product isolated was SP.

p-Bromospiroperidol was synthesized earlier in this laboratory and has been reported previously (4). A chlorinated SP analog was also prepared. The synthesis and characterization of this compound will be reported in more detail elsewhere (9).

Preparation of ^{77}Br

^{77}Br was prepared using the Argonne 60 inch cyclotron by the method described in a previous report (6). The separated ^{77}Br species was trapped in 0.5 ml 0.2% $Na_2S_2O_3$-0.2% NaOH. 2-3 mCi of ^{77}Br were obtained in typical runs.

Synthesis of ^{77}Br-p-bromospiroperidol (^{77}Br-BrSP)

A. Distillation Methods

Various oxidizing agents were added into a vessel containing ^{77}Br-bromide in 80% H_2SO_4. The radiobrominating species thus formed were distilled and swept into a trap containing SP in CCl_4-CH_2Cl_2 (1:1) kept at 0°C. The oxidants tested included $K_2Cr_2O_7$, Cl_2, NaOCl, Ce (IV), and $KMnO_4$.

After a set reaction time, the SP solution was washed with 1% $Na_2S_2O_3$-1% NaOH solution and then the solvent was evaporated off. The residue was dissolved in ethanol-H_2O (1:1) and analyzed by HPLC.

B. In Situ Methods

One pot reactions involving SP in glacial acetic acid and different oxidizing agents which generated the radiobrominating species in situ were investigated. The oxidizing agents tested were NaOCl, Chloramine-T, N-chlorosuccinimide, and H_2O_2.

The method utilizing H_2O_2 as oxidizing agent (10) was found to be the simplest and most reproducible. The following was adopted from the procedure reported by Duelfer et al. (11) for the H_2O_2 method and modified in this study to accommodate the other oxidants. 1 mg SP was dissolved in 100 ul glacial acetic acid. To this solution was added 200-500 uCi ^{77}Br in 100 ul dilute $Na_2S_2O_3$-NaOH solution. Finally, 50 ul 30% H_2O_2 (or a comparable amount of the other oxidizing agents) was introduced into the mixture. The reaction was allowed to proceed for a set period of time at room temperature after which the mixture was taken up with ethanol-water (1:1) to make a total volume of 2 ml. This was then purified by HPLC.

High Performance Liquid Chromatographic Separations

The ethanol-water solution of the above reaction mixture was injected directly into a preparative HPLC column. The ^{77}Br-p-bromospiroperidol peak was collected and evaporated down to 2 ml with a stream of nitrogen gas. This remaining solution was reinjected into an analytical column and the fraction containing the labelled product was collected. After removing the ethanol from the collected fraction by gentle evaporation, ^{77}Br-BrSP was extracted with an equal volume of CH_2Cl_2. The CH_2Cl_2 was finally evaporated off and ^{77}Br-BrSP residue was taken up in injectable saline.

An Altex HPLC system equipped with a UV detector and a radiation monitor was used in this study. A Rheodyne switching valve was added to the system to make it easier to direct the solvent flow through either a prepar-

ative column or an analytical column. The preparative column used was a Varian Micropak CH-10 50cm x 8mm column obtained from Varian Associates, Inc., Walnut Creek, Ca. while the analytical column was an RSil C-18 HL 25cm x 4.6mm column from Alltech Associates Inc., Deerfield, Il. The solvent for the preparative column was absolute ethanol: water: 4M ammonium acetate: glacial acetic acid (550: 427: 23: 10) while for the analytical column the same solvents were used but in different proportions (390: 595: 15: 10). Separations were done at room temperature and flow rates of about 1-2 ml/min were maintained. A 2 ml injection loop was utilized.

RESULTS AND DISCUSSION

We have previously prepared ^{77}Br-BrSP via the reaction of electrophilic ^{77}Br species, distilled from ^{77}Br-bromide in a mixture of concentrated H_2SO_4 and $K_2Cr_2O_7$, with ca. 50 ug SP in CH_2Cl_2-CCl_4 at dry ice temperature. Radiochemical yields in the range of 21-37% were obtained using this method but the consistency of this technique in terms of its success in labelling SP left much to be desired.

Various distillation methods utilizing several other oxidizing agents such as Cl_2, NaOCl, Ce(IV), and $KMnO_4$ were tried but these agents were found to offer no advantage over the H_2SO_4-$K_2Cr_2O_7$ method. Ce(IV) was expected to be a better oxidizing agent since it can selectively oxidize Br^- in the presence of Cl^- thus supressing chlorinating side reactions. This indeed was found to be true in several cold reactions involving microgram quantities of Br^- and SP where almost quantitative conversion into BrSP was observed. But under no carrier added conditions, no ^{77}Br-BrSP was found. More studies on optimal conditions are needed in order to utilize Ce(IV) as a selective Br^- oxidizing agent.

N-chlorosuccinimide (NCS) was one of the chlorine containing oxidants tested using in situ methods and is representative of this group of compounds.

Under the same conditions as the H_2O_2 reaction (see Methods Section), NCS induced formation of ^{77}Br-BrSP resulted in a radiochemical yield of 27% compared to 81% for the H_2O_2 reaction. Moreover, a large amount of what was later identified as a chlorinated SP was found in the NCS reaction. Under the conditions here, a third chromatographic separation was found necessary in order to obtain ^{77}Br-BrSP of acceptable purity. This step added another hour to the preparation time.

In the one-pot H_2O_2-induced reaction reported here, electrophilic ^{77}Br species formed in situ resulted into radiochemical yields of 80-90% which were consistently obtained within reaction times of 80-100 minutes at room temperature. These reaction times were found to be optimum as shown by the data found in Table 1.

TABLE 1: TIME DEPENDENCE OF THE REACTION $^{77}Br^- + H_2O_2 + SP$*

	Radiochemical Yields (%)		
Reaction Time (min)	^{77}Br	Unknown	^{77}Br-BrSP
30	91	ND†	9
40	57	ND	47
55	47	3	50
80	13	16	71
83	17	2	81
95	16	5	79
107	4	5	91
120	ND	19	81
160	6	26	68
170	ND	32	68

* Reaction Mixture: 1 mg SP + 100 ul glacial acetic acid + 100 ul ^{77}Br in dilute $Na_2S_2O_3$-NaOH + 50 ul 30% H_2O_2

† ND: Not Detected

The radiochemical yields listed in Table 1 were calculated from radiochromatograms typified by that shown in Figure 1. The unidentified labelled

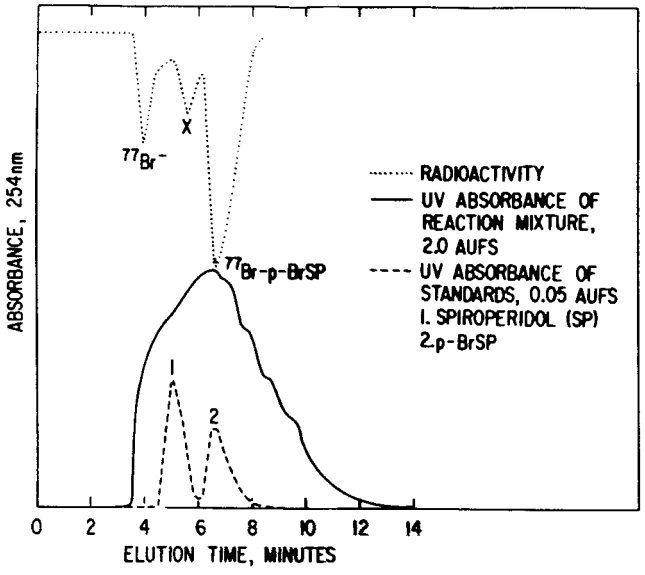


Figure 1: Reverse phase HPLC seperation of reaction mixture using a preparative column.

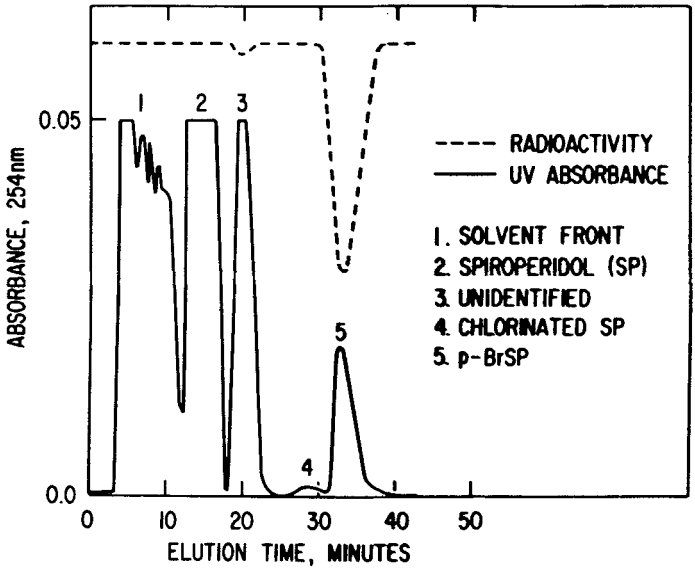


Figure 2: Rechromatography of ^{77}Br-p-BrSP peak from Figure 1 using an analytical column.

product appears to be the result of a secondary reaction that ^{77}Br-BrSP undergoes as suggested by the increase in the amount of this unknown product with time after the almost complete incorporation of ^{77}Br into SP. However, as can be seen in Figure 2, this unknown labelled product co-elutes with a large UV-absorbing peak which is possibly not the same compound. The stability of BrSP under these conditions was investigated by keeping cold BrSP in glacial acetic acid and H_2O_2 for two hours. Analysis of this mixture by HPLC failed to detect the unknown compound. Nonetheless, with reaction times of 80-100 minutes, the yield of ^{77}Br-BrSP can be maximized and the problem of the unknown product can hopefully be minimized.

The aqueous conditions used in the reaction made purification of the mixture by reverse phase HPLC more direct because of its compatibility with the HPLC solvent system. Moreover, the use of HPLC, compared to the previous use of thin layer chromotography (TLC), to purify and quantitate the product significantly reduced the preparation time and, in addition, insured that the ^{77}Br-BrSP product would be of high purity.

Because of the amount of substrate used (1 mg), a high capacity preparative column was found to be crucial to the efficient separation of the product. On the other hand since the small mass associated with BrSP would coelute with the tail of the large SP peak and, as shown in Figure 1, be inseparable from it, further purification with a low capacity but high efficiency analytical column has to be performed.

The need for a second separation is clearly illustrated in Figure 2 where it can be seen that a considerable amount of SP, and also the unknown compound, is still present after the first separation. Likewise, the presence of a by-product, identified to be a chlorinated SP, can be seen. A chlorinated SP (ClSP) is expected because of the ubiquity of Cl^-. Moreover, cold reactions involving microgram quantities of Cl^- and SP have shown that such a chlorinated

SP is produced under these conditions. Separating this by-product is important from the viewpoint of effective specific activity considering the fact that the biologic activity of this chlorinated analog may be different from SP or BrSP. This aspect is presently being studied in our laboratory.

Separation of BrSP by an analytical column also makes quantifying its mass a straight forward procedure. Although no Br^- carrier was added at any point in the synthesis, the highest specific activity of ^{77}Br-BrSP obtained using this procedure was 140 Ci/mmole at the end of the synthesis. The source of this Br^- contamination could be the As_2O_3 powder used as target material and/or the reagents used. It is interesting to note that the Cl^- contamination appears to be less than the amount of Br^- carrier present as seen by the smaller ClSP peak. However, in view of the observation by Senderoff et al. that under similar conditions the bromination of estradiol was at least 27,000 times faster than chlorination (12), the detection of a ClSP peak, though small, indicates the presence of a substantial amount of Cl^- contamination. Fortunately, the chloro analog can be separated by our HPLC method.

For the purpose of obtaining higher specific activity, ^{75}Br ($t_{1/2}$=100 min.), a shorter lived positron emitter useful for positron emission tomography (PET), would theoretically be the isotope of choice. A method of production of ^{75}Br using a small cyclotron has recently been reported (13) making this radionuclide more readily available. Preliminary studies adapting the procedure reported in this present work to the preparation of ^{75}Br labelled BrSP, now underway in our laboratory, show that higher specific activity, in the Ci/umole range, are indeed possible.

In conclusion, ^{77}Br labelled BrSP can be easily prepared via the reaction of ^{77}Br-bromide, H_2O_2, and SP in glacial acetic acid with reaction times of 80-100 minutes at room temperature. High SP concentrations and acidic conditions appear to be requirements for a successful labelling reaction. A two

column HPLC separation procedure was found necessary to ensure that the product is of high radiochemical purity and at the same time facilitate the quantitation of the product. ^{77}Br labelled BrSP is presently being used in our laboratory in studies assessing receptor binding affinities and densities in different animal models and the effects of specific activity.

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