Mechanistic approach of the nucleophilic $^{18}F^-$ exchange on 4'-NO_2- spiperone using TBA^{18}F or $K_{2.2.2.}/K^{18}F$(*).

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SUMMARY

High yield single step preparation of 4'-^{18}F-spiperone by $^{18}F^-$ for NO_2 exchange in highly basic conditions fails when using 4'-NO_2-spiperone as a precursor. This compound moreover inhibits radiofluorination of highly activated molecules in mentioned conditions. Inhibition is not provoced by ketalised analogues of the butyrophenone. This led to assume that enolisation of the butyrophenone was responsible for those phenomena. The assumption is supported by the fact that the results of inhibition experiments fit to a theoretical model.

Keywords : Fluoride-18, 4'-NO_2-spiperone, enolisation, inhibition.

INTRODUCTION

N.c.a. 4'-[^{18}F]-spiperone, structurally corresponding to the pharmaceutical spiperone, remains an attractive tracer for in vivo mapping of the dopaminergic area in the human brain using positron emission tomography . 4'-NO_2-spiperone was suggested by Killbourn et al (1, 2) as a suitable precursor for a one step preparation of 4'-[^{18}F]-spiperone, using Cs^{18}F or TBA^{18}F as a labelling agent. Unfortunately the overall yields obtained were not higher than 2%. A one step kryptofix-potassiumfluoride ($K_{2.2.2.}/K^{18}F$) mediated fluoride-18 for nitro exchange on 4'-NO_2-spiperone, showing a labelling yield of about 35%, was proposed by Hamacher et al (3).

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In our hands, pure 4'-NO_2-spiperone of two different sources failed to give this labelling yield, when using strong alkaline conditions such as the proposed $K_{2.2.2.}/K^{18}F$ method or when using n.c.a. $TBA^{18}F$ in DMSO.

The present study is an attempt to explain why 4'-NO_2-spiperone fails to give an efficient fluoride for nitro exchange in the proposed reaction conditions.

EXPERIMENTAL

Materials

DMSO was purified by distillation under reduced pressure over CaH and stored on 4 Å molecular sieves for further use. 1-Phenyl-1,3,8-triazaspiro[4.5]decan-4-one, γ-chloro-4-fluoro-butyrophenone, 4-chloro-1,1-ethylenedioxy-1-(4-nitrophenyl)butane and cyclopropyl-(4-fluoro-phenyl)ketone were purchased from Janssen Chimica - Beerse Belgium. Tetra-butylammoniumhydroxide (20% in water), di-kaliumoxalate monohydrate, kryptofix 2.2.2, 4'-fluoro-acetophenone were purchased from Merck-Schuchardt. γ-Chloro-p-nitro-butyrophe-none, cyclopropyl-p-nitro-phenylketone and 4'-nitro-spiperone were synthesized as described by Shiue et al (4). 4'-Nitro-spiperone was also obtained from the cyclotron departement of the university of Liege.

8-[4-(4-Fluorophenyl)-4-ethylenedioxy-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (9)(4)

1.6 mmol of 4-chloro-1,1-ethylenedioxy-1-(4-nitro-phenyl)butane, 1.6 mmol 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, 800 mg K_2CO_3 and 25 mg KI were heated at 100 °C in 10 ml DMF for 20 h, while gently stirring. After cooling, the reaction mixture was poured into 60 ml of water and extracted with EtOAc (2 x 40 ml). The organic phase was subsequently washed with water and dried with Na_2SO_4. After evaporating the solvent, the crude reaction product was purified on a Si-60 silica gel column and eluted with EtOH / $CHCl_3$(4/96 - v/v). White crystals were obtained after evaporation of the eluent and recrystallisation in isopropanol (yield : 82%). NMR (270 MHz, $CDCl_3$) d : 7.4 (dd, J = 4, 3 Hz ,2H); 7.3 (t, J = 8 Hz, 2 H); 7.0 (t, J = 9 Hz, 2 H); 6.9 (m, 3 H); 4.7 (s, 2 H); 4.0 (t, J = 7 Hz, 2 H); 3.8 (t, J = 7 Hz, 2 H); 2.7 (m, 6 H); 2.4 (t, J = 7 Hz, 2 H); 1.9 (m, 2 H); 1.56 -1.72 (m, 4 H).

4-Chloro-1,1-ethylenedioxy-1-(4-nitro-phenyl)butane (8)(5)

0.844 mmol γ-Chloro-p-nitro-butyrophenone, 2.56 mmol ethyleneglycol, 15 mg p-toluene sulfonic acid in 60 ml benzene were refluxed during 20 h in a flask equiped with a Dean α Stark

separator to collect the azeotropic distilled water. The reaction mixture was allowed to cool to room temperature and was extracted with 5% $NaHCO_3$ (3 x 25 ml) and water (3 x 25 ml). The organic phase was dried with Na_2SO_4 and the benzene was removed under reduced pressure. The crude reaction product was purified using a Si-60 silica gel column eluted with CH_2Cl_2/n-hexane (80/20 - v/v). Removing the eluent yielded 0.42 mmol (50%) of a pale yellow oil.

NMR (270 MHz, $CDCl_3$) d : 8.2 (d, J = 9 Hz, 2 H); 7.6 (d, J = 9 Hz, 2 H); 4.0 (t, J = 7 Hz, 2 H); 3.8 (t, J = 7 Hz, 2 H); 3.5 (t, J = 7 Hz, 2 H); 2.0 (m,2 H); 1.9 (m, 2 H)

8-[4-(4-nitro-phenyl)-4-ethylenedioxy-butyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one(10)(4)

The same reaction procedure as described for (8) was applied, resulting in pale yellow crystals (yield : 39%).

NMR (270 MHz, $CDCl_3$) d : 8.2(d, J = 9 Hz, 2 H), 7.6 (d, J = 9 Hz, 2 H);7.3 (t, J = 7 Hz, 2 H) 6.9 (m, 3 H); 4.7 (s, 2 H); 4.0 (t, J = 7 Hz, 2 H); 3.8 (t, J = 7 Hz, 2 H); 2.7 (m, 6 H);2.4 (t, J = 7 Hz, 2 H); 1.9 (m, 2 H); 1.5-1.7 (m, 4 H)

^{18}F-Production

$^{18}F^-$ was produced at the VUB CGR 560 cyclotron using the $^{16}O(\alpha, pn)^{18}F$ reaction. Bombarding 7 ml distilled water, contained in a stainless steel target, with 40 MeV α particles resulted in batches of 50 mCi $^{18}F^-$ after 1 hour irradiation at 12 μA. The target content was transferred to a polyethylene vessel via a 15 m polyethylene tubing. Besides the short lived ^{15}O, the irradiated water contained amounts of radioactive metal ions with $^{7}Be(t_{1/2} = 53.4$ d), $^{47}Sc(t_{1/2} = 3.42$ d), $^{48}V(t_{1/2} = 16.1$d) and ^{66}Ga ($t_{1/2} = 9.4$ h) as most important contaminants (6). This may point towards the presence of the motherions as impurities in the solution.

Labelling conditions

Basic

About 1 mCi of the aqueous fluoride-18 solution was added to an open platinum crucible containing 7,7 μmol TBAOH or 19 μmol K_2CO_3/40 μmol $K_{2.2.2}$. This mixture was dried at 115 °C using a mild nitrogen stream. The n.c.a. $^{18}F^-$ activity was recovered in 500 μl DMSO and added to a dry conical vessel containing 10 μmol of substrate. The vessel was tightly closed with a rubber stopper and metal screw cap and was heated at 150 °C in an oil bath during 15 minutes.

Neutral

When carrying out the nucleophilic exchange reaction with the less basic K_2oxalate in addition

to kryptofix, a mixture of 19 μmol $K_2C_2O_4.H_2O$ and 0.1 μmol K_2CO_3 was used (7).

Inhibition experiments

To 10 μmol p-NO_2-CPK, appropriate amounts of 4'-nitro-spiperone were added and the earlier described n.c.a. TBA^{18}F procedure was applied. After 15 minutes 50 μl samples were taken, quenched with 250 μl of HPLC-eluens and analysed.

Radiochemical purity of the $^{18}F^-$ solution

The radiochemical quality of each $^{18}F^-$ batch fluctuates due to varying amounts of contaminants (traces of metal ions, etc.). The suitability of the $^{18}F^-$ solution for use in nucleophilic radiofluorination reactions was checked by means of a reference reaction. p-NO_2-CPK was chosen for this purpose because of its high labelling characteristics and high stability in basic conditions. A labelling yield of at least 80% of p-^{18}F-CPK in neutral conditions was assumed to assure high enough radiochemical purity of the $^{18}F^-$ solution.

Quality control

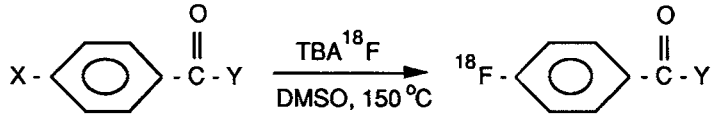
HPLC analysis was performed using a Hitachi 655A-11 chromatograph equipped with a Hitachi L-5000 LC controler, rheodyne injector (50 μl loop), Hitachi 655A UV monitor (254 nm), γ-scintillation detector (3" NaI (Tl) detector, Canberra electronics) and a Hitachi D-2000 Chromato-Integrator. Chromatography was carried out using an analytical Lichrospher RP-selekt B column (5 μm, Lichrocart 125-4) using a mixture of ACN/MeOH/0.01 M $(NH_4)_2HSO_4$/ TMA/HAc (10/35/55/0.2/0.4) at a flow rate of 1 ml/min. The radioactive peaks of interest were collected, quantified in a NaI γ–counter and expressed as a percentage of the amount injected.

RESULTS AND DISCUSSION

Although NO_2 is assumed to be a more suitable leaving group than fluoride (8), the radiofluorination reaction of 4'-F-acetophenone (3) gives higher labelling yields than for 4'-NO_2-acetophenone (2) (table 1). These results are in agreement with those obtained by Shiue et al (9). More dramatically are the results obtained with spiperone analogues(4 and 5), where introduction of a NO_2 function in stead of fluoride decreases the labelling yield from 28% to about 0%.

Table 2 shows the influence of different compounds on the labelling yield of the reference reaction (1), using n.c.a. TBA^{18}F as a labelling agent. Adding an equimolar amount of 1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one (6, spirohead) to the nucleophilic radiofluorination reaction of p-NO_2-CPK, allows to obtain labelling yields exceeding 90%. This means that this

TABLE 1 : $^{18}F^-$ for NO_2 exchange on different keto-aryl compounds(**).



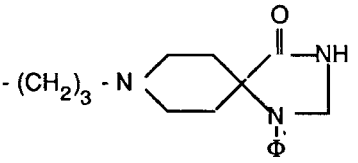
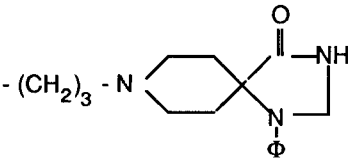
	X	Y	Labelling yield %
1	NO_2		90
2	NO_2	$-CH_3$	52
3	F	$-CH_3$	80
4	NO_2		0
5	F		28

TABLE 2 : Influence of different compounds on the radiofluorination reaction of p-NO_2-CPK(**).

	compound added	% p-^{18}F-CPK
6	spirohead	93
7	γ-Cl-p-NO_2-butyrophenone	0
8	ketal of γ-Cl-p-NO_2-butyrophenone	54 (+ 42)
4	4'-F-spiperone	63 (+ 13)
9	ketal of 4'-F-spiperone	90
5	4'-NO_2-spiperone	0
10	ketal of 4'-NO_2-spiperone	90

(**) The results in table 1 and 2 are the mean of at least 5 experiments.

part of the spiperone molecule does not contribute to the inhibition of the radiofluorination reaction.

A complete inhibition of the radiofluorination of p-NO_2-CPK is observed, when adding γ-Cl-p-NO_2-butyrophenone (7) or 4'-NO_2-spiperone (5) to the reaction mixture. Important was the fact that the corresponding ketals of these compounds (8 and 10) no longer inhibited the nucleophilic exchange. For the ketal form of 4'-F-spiperone (9) (F desactivated for exchange) and 4'-NO_2-spiperone (10), 90% of the reference substrate is labelled, while in presence of the ketal form of γ-Cl-p-NO_2-butyrophenone (8) almost 96% of the available radiofluoride is consumed, partially 54% for the labelling of the reference substrate, partially 42% for fluoride for γ-chloro exchange in the alkyl side chain. In case of 4'-F-spiperone (4), p-NO_2-CPK is radiofluorinated for 63% and 13% of the radiofluoride is used for isotopic exchange. 14% of the free fluoride-18 is apparantly not available for reaction, again pointing towards a trapping of fluoride-18 in the reaction mixture.

A mean labelling yield of 80 % was obtained for the reference substrate when applying "neutral" conditions, while alkalic conditions allow to obtain 90%. In neutral conditions 4'-NO_2-spiperone was labelled with a yield ranging from 13 -18%. Corrected for the actual reference yield, this means that 17% - 24% of the available radiofluoride is exchanged. The lower labelling yields are due to the formation of metal-fluoride complexes, as in strong alkaline conditions the metal ions are present in their hydrolysed form.

ENOL-CAPTURE HYPOTHESIS

The results mentioned above led to the hypothesis, that in strong basic conditions, an enol tautomer of 4'-NO_2-spiperone is generated, resulting in the capture of the free fluoride-18 ions due to formation of hydrogen bonds. Enol formation coupled to inhibition must be seen on the micro-scale of the occurring labelling reaction. Suppose 0.1% of the 4'-NO_2-spiperone is converted to enol, then this represents 2.10^{-5} M in the reaction conditions used. The currently available NMR-techniques with a detection limit of abouyt 10^{-4} M do not allow to give quantitative confirmation of such low amounts of enol. The specific activity of n.c.a. $^{18}F^-$ is 6.3 10^{13} MBq/mol. In the applied reaction conditions 37 MBq corresponds to ± 10^{-9} M. This means an excess of 10^4 enol over $^{18}F^-$.

The results of the inhibition of the radiofluorination reaction of p-NO_2-CPK by 4'-NO_2-

spiperone(figure 1) are fitted to a simple theoretical model of the enol capture hypothesis premised.

When labelling occurs in presence of inhibitor, two additional reactions are supposed to occur :

1. enolisation, governed by the equilibrium constant K, equal to the enol to keto ratio

$$\text{keto} \rightleftharpoons \text{enol} \qquad K = \frac{[\text{enol}]}{[\text{keto}]} \qquad (1)$$

2. capture of the free $^{18}F^-$ ions represented by the dissociation constant K_D at equlibrium

$$\text{enol} + {}^*F^- \rightleftharpoons \text{enol}^*F \qquad K_D = \frac{[\text{enol}]\,[{}^*F^-]}{[\text{enol}^*F]} \qquad (2)$$

As [enol] >> [$^*F^-$], it can be assumed that [enol] ~ $[\text{enol}]_o$ and $[{}^*F]'_o = [{}^*F] + [\text{enol}^*F]$ *(3)*, with $[{}^*F^-]'_o$ the initial fluoride concentration involved in the labelling reaction in absence of inhibitor.

(3) in *(2)*

$$\frac{1}{K_D} = \frac{1}{[\text{enol}]} \left\{ \frac{[{}^*F^-]'_o}{[{}^*F^-]} - 1 \right\} \qquad (4)$$

(1) x *(4)*

$[{}^*F^-]/[{}^*F^-]'_o$ corresponds to the ratio $\eta_i/\eta = N$ were η_i and η are respectively the labelling yields in presence and absence of inhibitor.

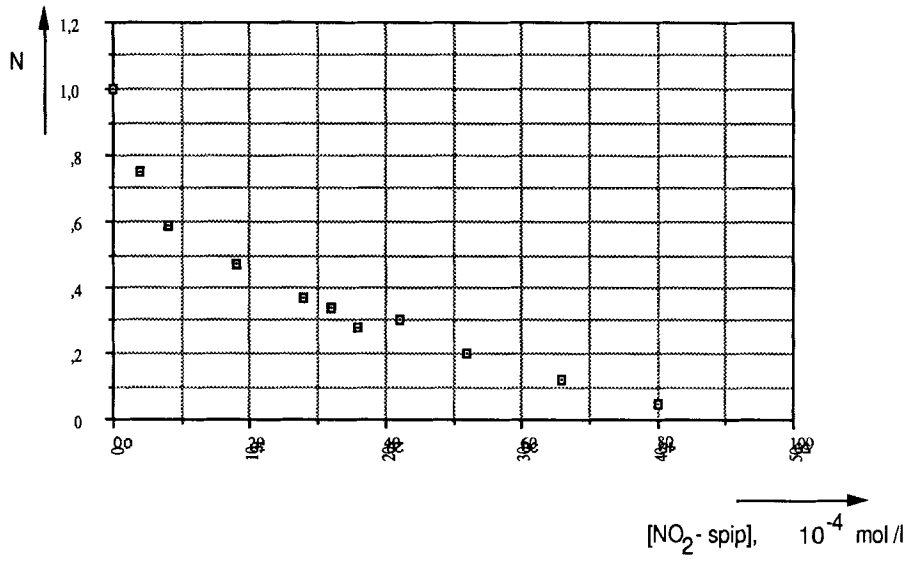
$$\frac{[{}^*F^-]'_o}{[{}^*F^-]} = 1 + \frac{K}{K_D}\,[\text{keto}]_o \qquad (5)$$

or

$$\frac{[{}^*F^-]}{[{}^*F^-]'_o} = \frac{K_D}{K_D + K[\text{keto}]_o} \qquad , [\text{keto}]_o = \text{initial concentration} \qquad (6)$$

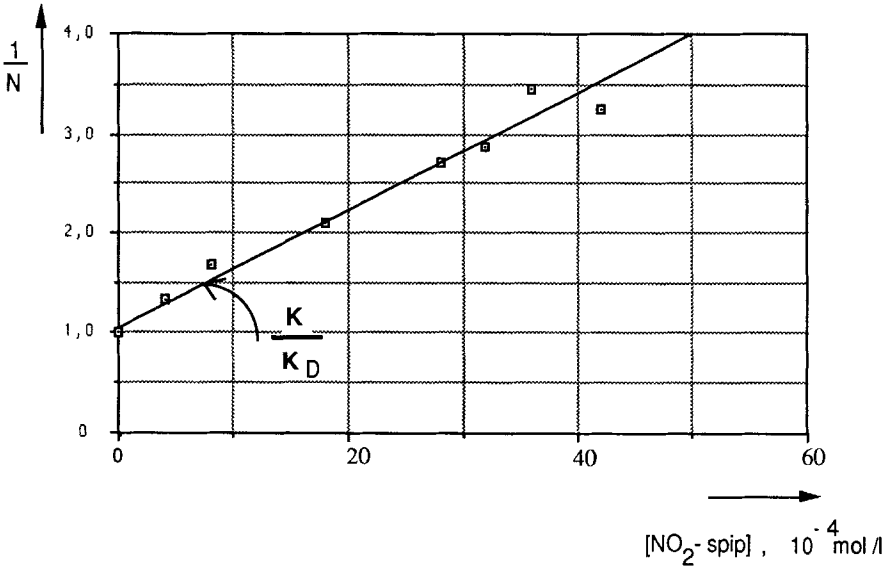
When plotting the experimental values of N as a function of [NO_2-spip], the ketone added, a hyperbolic like curve is obtained .

Figure1 : Inhibition by 4'-NO_2-spiperone of the radiofluorination of p-NO_2-CPK



Plotting 1/N as a function of [NO_2-spip], corresponding to equation *(5)*, yields a straight line with the slope K/K_D ~ 5,85 . 10^2 M .

Figure 2 : 1/N as a function of [NO_2 -spip]



This proves that the experimental data fit well with the theoretical model of enol formation and inhibition.

CONCLUSION

As a conclusion we can state that 4'-nitro-spiperone is not suitable as a substrate for the preparation of 4'-[^{18}F-]-spiperone using highly basic conditions, but can be used in "neutral" conditions. Nevertheless neutral labelling conditions require highly pure radiofluoride solutions free from metalic ion contaminants. The extreme low labelling yield in basic conditions is assumed to be due to the formation of an enol. This assumption is supported by inhibition experiments and a theoretical fit.

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