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SYNTHESIS, QUALITY CONTROL AND TISSUE DISTRIBUTION OF 2-[^{18}F]-NICOTINIC ACID DIETHYLAMIDE, A POTENTIAL AGENT FOR REGIONAL CEREBRAL FUNCTION STUDIES

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2-[^{18}F]-nicotinic acid diethylamide was prepared by nucleophilic aromatic substitution in an acetamide melt of the corresponding chloro-compound and purified by high pressure liquid chromatography. Optimization of the reaction conditions led to a maximum radiochemical yield of about 50% within less than one half-life of ^{18}F. Tissue distribution of 2-[^{18}F]-nicotinic acid diethylamide in various organs of mice showed a very fast accumulation of activity in the brain (mean body concentration MBC = 239%) with a brain to blood ratio of 1.34.

Introduction

Syntheses of aromatic ^{18}F-labelled compounds are generally carried out by one of the following two methods: (i) fluorination by molecular $^{18}F-F_2$ or ^{18}F-atoms and (ii) fluorination via the Balz-Schiemann reaction. In general, reactions of organic compounds with F_2 occur in a very violent and unselective way, leading to a wide product spectrum. Several attempts, however, have led to a reduction of reactivity by improvements of the experimental techniques.[1] Interesting effects of substrate selectivity and reactivity in aromatic substitution reactions by elemental fluorine have been found by CACACE and WOLF.[2] ROWLAND et al. studied the fluorination by homolytic aromatic substitution in aromatic ^{18}F-systems after the $^{19}F(n, 2n)^{18}F$ reaction.[3,4] However, for practical purposes or for

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the application to more complex molecules these methods cannot be applied due to low yields, uncontrolled side reactions and/or radiation damage in recoil systems. In contrast to most of the fluorination reactions by molecular or atomic halogen the BALZ–SCHIEMANN reaction allows the introduction of a fluorine atom into a definite position of an aromatic molecule. This classical method[5] is described in many later publications indicating a considerable dependence of the yields on different reaction parameters like substrate, temperature, solvent etc.[6] Application of this method for the synthesis of aromatic ^{18}F-labelled compounds, however, results in products with low specific activities due to the amount of fluorine carrier in the BF_4-reagent and/or poor radiochemical yields.[7–13]

A modification of the BALZ – SCHIEMANN reaction is the decomposition of piperidyl triazenes leading to simple ^{18}F-labelled aryl fluorides with high radiochemical yields.[14] Application of this method to more complex molecules, however, was found to give very low product yields indicating a strong dependence of the reaction on the nature of the intermediate triazene.[15]

I-for-halogen exchange in the melt of acetamide and an aromatic halogen-containing substrate has been described by ELIAS and LOTTERHOS.[16] Nucleophilic fluorination of aromatic molecules, however, should only occur in strongly desactivated compounds in which F-for-halogen substitution is favoured by electron-withdrawing substituents and/or nitrogen in heterocycles.

In this work we describe the fluorination of a pyridine derivative by nucleophilic substitution in the melt of acetamide. The non-halogenated compound nicotinic acid diethylamide is known as a centrally acting analepticum influencing the activity of heart and respiration and is applied for patients suffering from chronic insufficient respiration. Patients' and metabolic studies of this pharmaceutical have been described in the literature.[17–22] With respect to the above mentioned effects, the analogous ^{18}F-labelled compound was investigated with the aim of its potential application in regional cerebral function studies by positron emission tomography.

Experimental

Materials

KF and acetamide were purchased from Merck (Darmstadt) with a purity of >99%. The acetamide was further purified by recrystallization from benzene and then dried over P_2O_5. 2-Cl-nicotinic acid and 2-F-nicotinic acid diethylamide (B.P. 112 °C; 0.2 mm) were obtained from Emka-Chemie (Markgröningen). The diethylamide of 2-Cl-nicotinic acid was prepared in our laboratory by classical methods, i.e. transformation of the acid into the acid chloride followed by the reaction

with diethylamine in dioxane, extraction with diethylether from aqueous alkaline solution and vacuum distillation of the product.

Irradiation

^{18}F was produced via the $^{16}O(^3He, p)^{18}F$-reaction at the Jülich Compact Cyclotron CV 28 using a water target. The water was of ultra high quality (AMPUWA, E. Fresenius KG, Bad Homberg). About 10 ml of water circulating through the target were irradiated with a ^3He-beam (36 MeV, 10 μA) for about 20 minutes and 20 to 30 mCi of "carrier-free" ^{18}F in aqueous solution were obtained.

Synthesis

To the ^{18}F-containing water in a quartz bulb KF-carrier was added (except for the carrier-free synthesis). The solution was evaporated to dryness in a He-stream at 150 °C. The drying process was completed by heating the bulb at 250 °C for 20 minutes. After cooling, 2-Cl-nicotinic acid diethylamide ($\rho \cong 1.26$ g/ml) and 100 mg acetamide were added, the bulb was sealed with a teflon collar and a glass stopper and heated in an oil bath while stirring. After a given reaction time the contents of the bulb was transferred into a 60 ml extraction vessel by washing with 10 ml of water, adding 5 ml of aqueous NaOH-solution (1 : 1) and cooling the warm solution in an ice-bath. The organic products were extracted 3 times with diethylether (1X10 ml, 2X5 ml each). After evaporation to dryness, the residue was dissolved in 2 ml of diethylether and 2-[^{18}F]-nicotinic acid purified by high pressure liquid chromatography (hplc). Conditions for the hplc-separation were as follows: Column: Lichrosorb Si 60 (7 μm), 50 cm long, 1.6 cm i.d.; eluents: diethylether; flow: 11 ml/min; pump: Orlitta with pulsation damper; detector: Knauer dualdetector (UV and RI). k′-values: 2-F-nicotinic acid diethylamide $k' = 4.4$; 2-Cl-nicotinic acid diethylamide $k' = 6.1$. Measuring the radioactivity with a well-type NaI(Tl) scintillation crystal 2-[^{18}F]-nicotinic acid diethylamide could be separated as main product from small amounts of ^{18}F-labelled impurities. Although the k′-values were influenced by the excess amount of 2-Cl-nicotinic acid diethylamide, a good separation of 2-[^{18}F]-nicotinic acid diethylamide was obtained.

Animal experiments

For animal experiments, the eluate of the product fraction after hplc-separation was evaporated to dryness, carrier-free 2-[^{18}F]-nicotinic acid diethylamide dissolved in 2–3 ml isotonic saline solution at 60 °C and the solution sterilized by Millipore filtration. Aliquots of 100 μl with activities ranging from 1–10 μCi

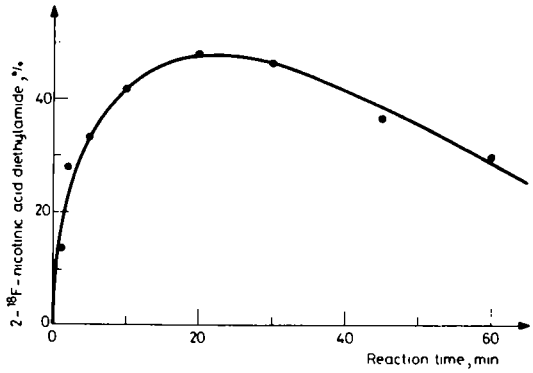

Fig. 1. Time dependence of F-for-Cl exchange in the 2-Cl-nicotinic acid diethylamide system. (Reaction conditions: 1 mg KF, 100 mg acetamide, 50 µl 2-Cl-nicotinic acid diethylamide, 250 °C). Average deviations are within ±10%

2-[^{18}F]-nicotinic acid diethylamide were injected into the tail vein of female NMRI albino mice (body weights between 27 and 33 g). After given time intervals the animals were killed, the organs removed, blotted dry of blood, counted in a well-type NaI(Tl) scintillation crystal, and weighed. The results are expressed in terms of % mean body concentration (MBC) in the particular organ. This value is obtained by the expression: MBC(%) = specific activity of the organ (cpm/g organ) divided by the applied dose (cpm/g body weight) and multiplied with 100.

Results and discussion

Synthesis

Fig. 1 shows the time dependence of the radiochemical yield of 2-[^{18}F]-nicotinic acid diethylamide. It can be seen that within the first minutes a fast increase of the yields takes place reaching its maximum value of about 50% between 15 and 30 minutes followed by a slower decrease. The reaction was carried out at a temperature of 250 °C. With increasing time a slight yellow colour of the melt indicates decomposition of the starting material and probably of the ^{18}F-labelled product, too, as can be seen by the decreasing yields at reaction times above about 30 minutes. A similar effect can be observed for the temperature dependence of the radiochemical yields (Fig. 2). At a constant reaction time of 30 minutes the optimum yield was obtained at 250 °C, followed by a fast decrease. At 300 °C practically no product was formed and the tarry contents of the bulb

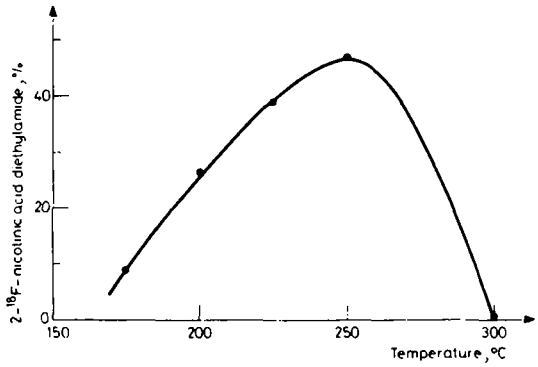

Fig. 2. Temperature dependence of F-for-Cl exchange in the 2-Cl-nicotinic acid diethylamide system. (Reaction conditions: 1 mg KF, 100 mg acetamide, 50 µl 2-Cl-nicotinic acid diethylamide, 30 min reaction time). Average deviations are within ±10%

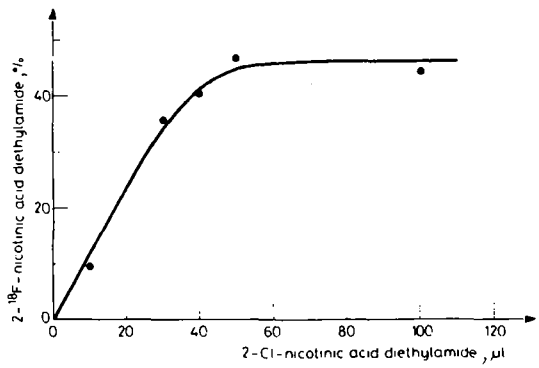

Fig. 3. Substrate dependence of F-for-Cl exchange in the 2-Cl-nicotinic acid diethylamide system. (Reaction conditions: 1 mg KF, 100 mg acetamide, 30 min reaction time at 250 °C). Average deviations are within ±10%

indicated considerable decomposition. Finally, Fig. 3 shows the yields of 2-[^{18}F]-nicotinic acid diethylamide as depending on the amount of the starting material. At 50 µl 2-Cl-nicotinic acid diethylamide a saturation yield of about 50% is reached. Obviously, this amount of the starting compound is necessary to obtain reasonable radiochemical yields. A further increase, however, does not influence the exchange.

An interesting effect is observed when studying the yields of the F-for-Cl exchange reaction by variation of fluorine carrier. The results are shown in Fig. 4.

Table 1

Tissue distribution in mice of 2-[^{18}F]-nicotinic acid diethylamide

Organ time, min	n=4 0.1	n=4 0.25	n=12 0.5	n=12 0.75	n=12 1.0
Brain	1.50±0.18	2.39±0.22	1.77±0.20	1.67±0.16	1.49±0.15
Blood	1.71±0.18	1.78±0.40	1.45±0.20	1.58±0.10	1.41±0.09
Kidneys	2.17±0.34	1.14±0.30	1.48±0.43	1.53±0.12	1.44±0.11
Liver	1.59±0.23	0.69±0.19	1.40±0.26	1.71±0.24	2.12±0.20
Intestine	0.55±0.16	0.44±0.10	0.73±0.22	0.87±0.09	0.83±0.10
Heart	1.96±0.35	1.92±0.41	1.59±0.26	1.69±0.15	1.46±0.09
Lung	2.60±0.06	2.28±0.37	2.14±0.66	2.49±0.50	2.06±0.38
Fat		0.36±0.07	0.38±0.15	0.40±0.09	0.50±0.13
Pancreas		0.84±0.14	1.21±0.18	1.32±0.14	1.24±0.14
Thymus		1.53±0.27	1.25±0.17	1.32±0.17	1.21±0.30
Spleen		0.43±0.20	0.74±0.12	0.82±0.16	0.87±0.14
Muscle		1.46±0.20	1.32±0.17	1.32±0.22	1.10±0.24
Uterus		0.67±0.18	0.64±0.24	0.62±0.25	0.90±0.13
Skin		0.52±0.12	0.47±0.15	0.65±0.11	0.69±0.10
Glandulae submax.		2.75±0.26	1.98±0.35	1.92±0.71	1.87±0.19

Values expressed as 1/100% MBC (see text).
Values are means ±S.D.

Reduction of the concentration of potassium fluoride over a range of six orders of magnitude influences the radiochemical yields of 2-[^{18}F]-nicotinic acid diethylamide only to a small extent. Even in the case of a no-carrier-added synthesis yields up to 40% were obtained. Measuring the sensitivity of the UV-detector for 2-F-nicotinic acid diethylamide a detection limit of $5 \cdot 10^{-4}$ mg ($\cong 2.6 \cdot 10^{-9}$ Mol) was found. However, it can be assumed that the fluorine impurities in the chemicals are even lower leading to a specific activity of at least 20 Ci/mg at the end of the synthesis. The possibility of a „no-carrier-added" synthesis is an advantage to ^{18}F-labelling methods of long-chain fatty acids where a drastic reduction of the yields with decreasing amounts of fluoride-carrier takes place.[2,3]

Animal experiments

As expected, accumulation of activity in the first seconds after injection is found in all well-perfused organs like brain, lungs, heart, and kidneys (see Table 1). A correlation between values %MBC and %dose/g organ is given by the relation-

at different times after i.v. injection (n = number of animals)

n=4 1.25	n=12 1.50	n=12 2.0	n=12 3.0	n=8 5.0	n=4 10.0
1.64±0.09	1.27±0.18	1.30±0.17	1.20±0.08	1.06±0.07	1.01±0.06
1.51±0.16	1.29±0.12	1.38±0.14	1.35±0.10	1.27±0.11	1.19±0.06
1.54±0.08	1.41±0.08	1.49±0.10	1.63±0.24	1.46±0.20	1.39±0.06
1.88±0.18	2.04±0.19	2.28±0.39	2.21±0.21	2.09±0.31	2.02±0.12
0.87±0.11	0.89±0.13	0.92±0.12	0.98±0.10	1.04±0.11	1.14±0.08
1.54±0.06	1.33±0.13	1.42±0.15	1.36±0.12	1.21±0.12	1.11±0.07
2.02±0.04	2.02±0.31	2.12±0.19	2.03±0.29	1.59±0.16	1.38±0.09
0.57±0.10	0.61±0.09	0.63±0.14	0.53±0.19	0.68±0.15	0.66±0.02
1.30±0.10	1.30±0.09	1.25±0.16	1.16±0.10	1.13±0.07	1.08±0.08
1.30±0.07	1.13±0.18	1.21±0.21	1.18±0.08	1.11±0.11	1.07±0.06
0.93±0.24	1.07±0.10	1.15±0.16	1.14±0.12	1.19±0.10	1.12±0.08
1.40±0.13	1.18±0.17	1.17±0.15	1.24±0.13	1.11±0.06	1.00±0.12
0.95±0.10	0.91±0.13	1.07±0.23	1.11±0.12	1.23±0.07	1.19±0.16
0.64±0.09	0.76±0.17	0.80±0.07	0.76±0.07	0.81±0.05	0.84±0.05
2.59±0.84	2.13±0.24	2.78±0.88	2.46±0.24	2.06±0.49	1.58±0.13

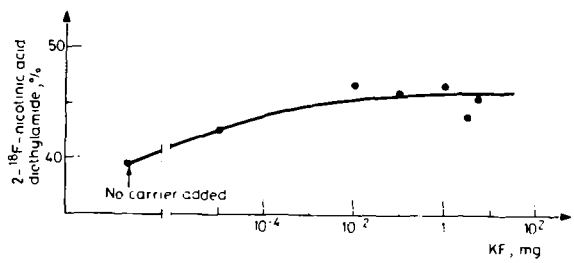

Fig. 4. Carrier dependence of F-for-Cl exchange in the 2-Cl-nicotinic acid diethylamide system. (Reaction conditions: 100 mg acetamide, 50 µl 2-Cl-nicotinic acid diethylamide, 30 min reaction time at 250 °C). Average deviations are within ±10%

ship 30% MBC ≅ 1% dose/g organ due to the mean weight of a mouse (30 g). Already 15 seconds after injection, a maximum of 239 %MBC ≅ 8% dose/g organ is accumulated in the brain decreasing within the following 10 minutes. At this time the brain/blood ratio is 1.34 compared to 1.08 for that of the ratio heart/blood (see Figs 5, 6 and 7). By separation of the brain into two fractions (a) cerebrum and (b) cerebellum and brain stem no significant difference in activity distribution could be observed. Excretion of activity occurs partially by the kidneys

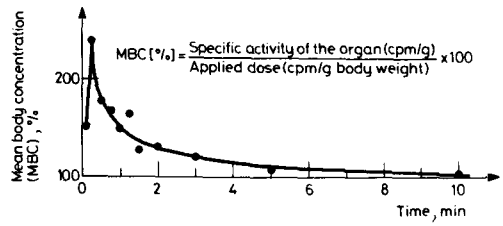

Fig. 5. Time course of radioactivity in brain of mice after i.v. injection of 2-[^{18}F]-nicotinic acid diethylamide (100 µl of isotonic saline solution containing 1–10 µCi 2-[^{18}F]-nicotinic acid diethylamide). Deviations see Table 1

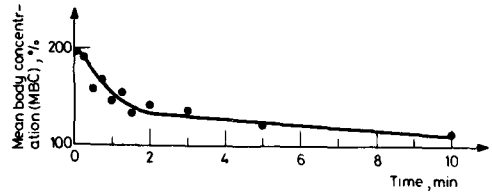

Fig. 6. Time course of radioactivity in heart of mice after i.v. injection of 2-[^{18}F]-nicotinic acid diethylamide (100 µl of isotonic saline solution containing 1–10 µCi 2-[^{18}F]-nicotinic acid diethylamide). Deviations see Table 1

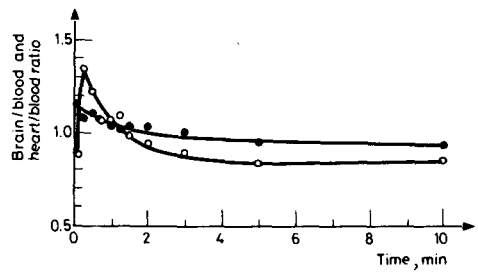

Fig. 7. Time course of the ratios brain/blood (-o-o-) and heart/blood (•) in mice after i.v. injection of 2-[^{18}F]-nicotinic acid diethylamide (100 µl of isotonic saline solution containing 1–10 µCi 2-[^{18}F]-nicotinic acid diethylamide)

and to a larger extent by the liver as can be seen in Table 1 by an increase of liver activity within the first 2 minutes after injection, followed by a delayed increase in intestine. Due to the high heart rate in mice, the fast maxima of accumulation after injection and the rate of metabolism can be explained. For an application in larger animals or in man the maxima should be expected within about one hour after injection.

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