Preparation of N(1-ethyl-2-pyrrolidyl-methyl)2-methoxy-4-iodo-^{125}I-5-ethyl sulfonyl benzamide: a radioligand for the radioimmunoassay of sulpiride-related compounds

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SUMMARY

We describe the preparation of ^{125}I labelling with a higher specific radioactivity of N(1-ethyl-2-pyrrolidyl-methyl)2-methoxy-5-ethyl sulfonyl benzamide, a potent biological analogue for sulpiride. The incorporation of iodine in the molecule was achieved by the substitution of aromatic amino groups via the diazo compound. Binding and immunological parameters of iodinated and tritiated tracers were compared.

KEY WORDS : sulpiride, radioimmunoassay, ^{125}I iodination

INTRODUCTION

Radioimmunoassay (RIA) is a precious analytical tool for the study of the metabolism and pharmacokinetics and for clinical monitoring of psychoactive drugs (1).

The use of a tritiated tracer with a high specific radioactivity (up to 30 Ci/mmole) provides in most cases a very sensitive test. However, an iodine ^{125}I labelled radioligand offers the advantages of greater sensitivity related to higher specific radioactivity, simplicity of preparation and more convenient and economical counting procedures (2).

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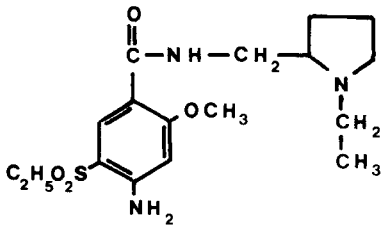
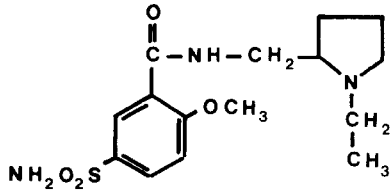
We describe here the synthesis of N(1-ethyl-2-pyrrolidyl-methyl)-2-methoxy-4-iodo-^{125}I-5-ethyl sulfonyl benzamide, which is used as tracer in the RIA of N(1-ethyl-2-pyrrolidyl-methyl)2-methoxy-4-amino-5-ethyl sulfonyl benzamide (DAN 2163), a potent pharmacological analogue of sulpiride (3).



DAN 2163



Sulpiride

MATERIALS AND METHODS

Chemicals

DAN 2163, sulpiride and their related compounds are kindly supplied by Delagrange (France).

Tritiated DAN 2163 (30 to 50 Ci/mmole), labelled at the level of the ethyl group of the pyrrolidyl ring, was provided by Commissariat à l'Energie Atomique, Département de Biologie, Service des Molécules Marquées, CEN Saclay (France).

$Na^{125}I$, carrier-free sodium iodide was purchased from CEA (France), and all the other materials are of the analytical grade.

Preparation of ^{125}I labelled compound

One iodine atom was incorporated in the benzene ring of DAN 2163 by Sandmeyer's reaction. One nmole (10 µl) of DAN 2163 in HCl N was diazoted with 1 nmole (1 µl) of Na NO_2. The reaction tube was left in an ice bath. 2 µl (approx. 400 µ Ci) of Na ^{125}I were added immediately and the reaction was carried out 12h at 22° C in darkness. Purification of the labelled substance was performed by thin-layer chromatography (TLC) on silica gel plate in Di-isopropyl ether-ethanol-ammonia (65 : 25 : 10) upper phase, as solvent system. Radioactive spot was localized by autoradiography (Rf = 0.60) and the substance was eluted from the silica with methanol and stored at -20° C until use.

Preparation of ^{127}I DAN 2163

The schedule for ^{127}I iodination of DAN 2163 was the same as that described above, using 10^4 times more materials:10 µmoles of DAN 2163 in HCl N (500 µl) were diazoted with 10 µmoles of $NaNO_2$ (100 µl).Ten umoles of Na^{127}I (100 µl) were added immediately and the reaction was carried out overnight at room temperature.The cold iodinated substance comigrating with ^{125}I radioactive substance was eluted from the silica by methanol.

Radioimmunological procedure

In polystyrene test tubes were placed one after another 100 µl of standard or RIA buffer (phosphate buffer 0.1 M pH 7.4, 0.5% bovine serum albumin), 100 µl of ^{3}H-DAN or iodinated tracer (approx. 5,000 cpm) and 100 µl of diluted antiserum. Each tube was duplicated.

The reaction was left one night at +4° C. The bound and free fractions of tracer were separated by adding 1 ml of cold n-propanol. The tubes were immediately vortexed and centrifuged for 15 min. at +4° C. The radioactivity of the supernatant(^{3}H)or pellet(^{125}I) respectively, was measured.

RESULTS AND DISCUSSION

The method for conversion of the diazo group into halogen was originally described by Sandmeyer, using cuprous chloride as catalyst for decomposition of the diazo chlorides.

In this way the iodine derivative may be prepared by adding sodium iodide to a solution of the diazonium salt containing excess mineral acid and gently warming (4). This reaction has, to our knowledge, never been described in the literature as applied for ^{125}I labelling of an aromatic ring with higher specific radioactivity using carrier-free Na ^{125}I. ^{125}I iodination of DAN 2163 using oxydation of Na ^{125}I by chloramine T (5) failed either due to deactivation of the C_3 carbon of the aromatic ring or due to steric hindrance. In our standard procedure we expect that the iodine atom will become linked to the C_4 carbon. In agreement with this hypothesis, the cold ^{125}I iodinated substance cannot be diazotized suggesting the lack of aromatic amino group. Complete separation on silica gel plate of iodinated substance from diazo compound and unreacted DAN 2163 allowed us to obtain for the radioactive tracer the specific radioactivity of the iodide precursor.

A comparison of the binding of ^{3}H-DAN 2163 and ^{125}I-DAN 2163 with antibodies is shown in Figure 1. The increase of titer observed with the iodinated tracer suggests that this substance has a similar structure than the antigenic determinant. Coupling of DAN 2163 with the antigenic carrier was carried out by diazoted DAN 2163 and that is probably why structural modification at the level of the position of aromatic carbon C_4 in the DAN 2163 tracer molecule does not modify the recognition by the antibody site. The higher specific radioactivity of the iodinated tracer permits a considerable reduction in tracer mass and antiserum dilution.

Figure 2 shows the standard curves obtained with tritiated or iodinated tracers. Increased sensitivity was obtained when ^{125}I-DAN 2163 was used instead of ^{3}H DAN 2163. This result was predictable in view of the much lower specific radioactivity of the latter and the high affinity of iodinated tracer for the binding site. On the basis of these results we are carrying out pharmacokinetic studies of DAN 2163 in human subjects, and the high sensitivity of the test allowed us to assay sulpiride and other related compounds which cross-react with antibodies.

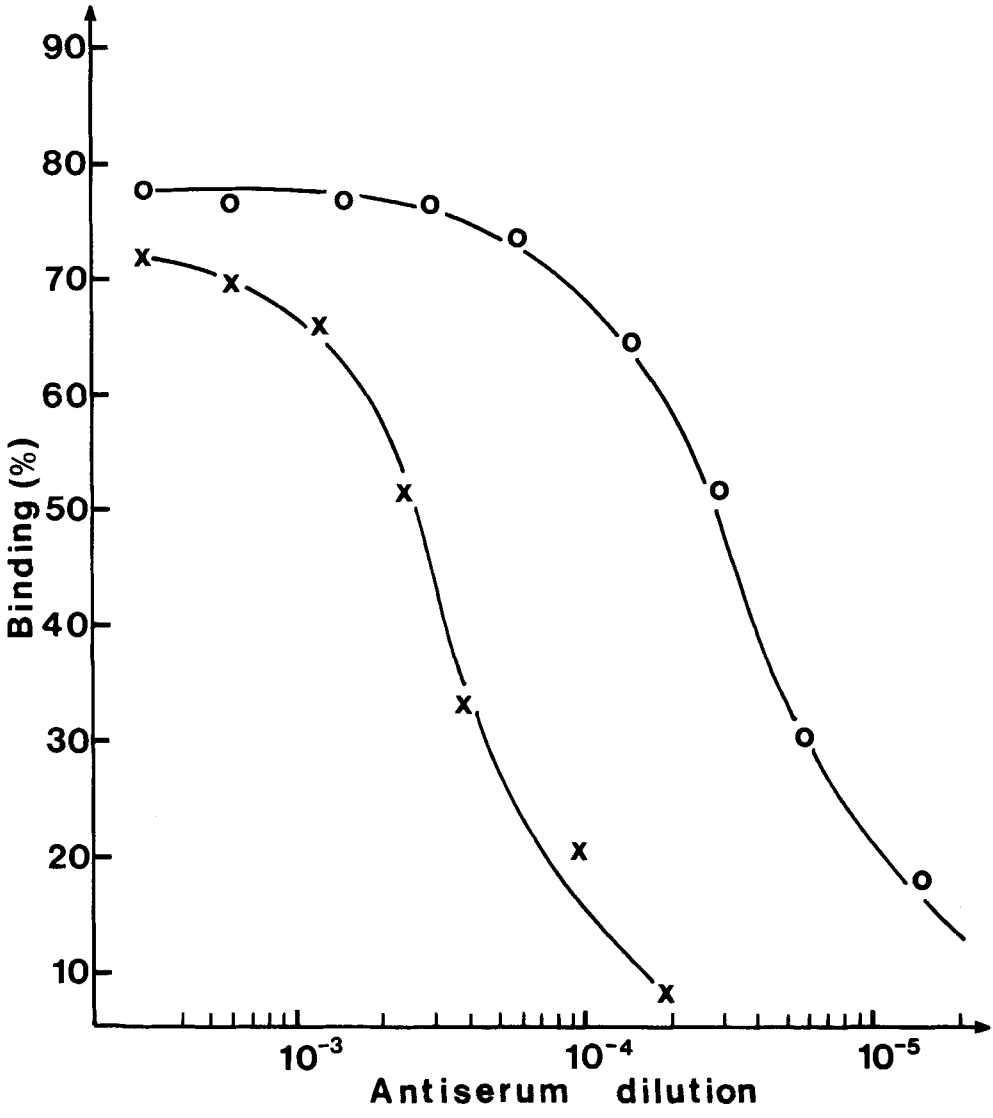


Figure 1

Comparison of the binding of labelled DAN 2163 with anti DAN 2163 antiserum using ^{3}H-DAN 2163 (X--X) and ^{125}I-DAN 2163 (O--O).

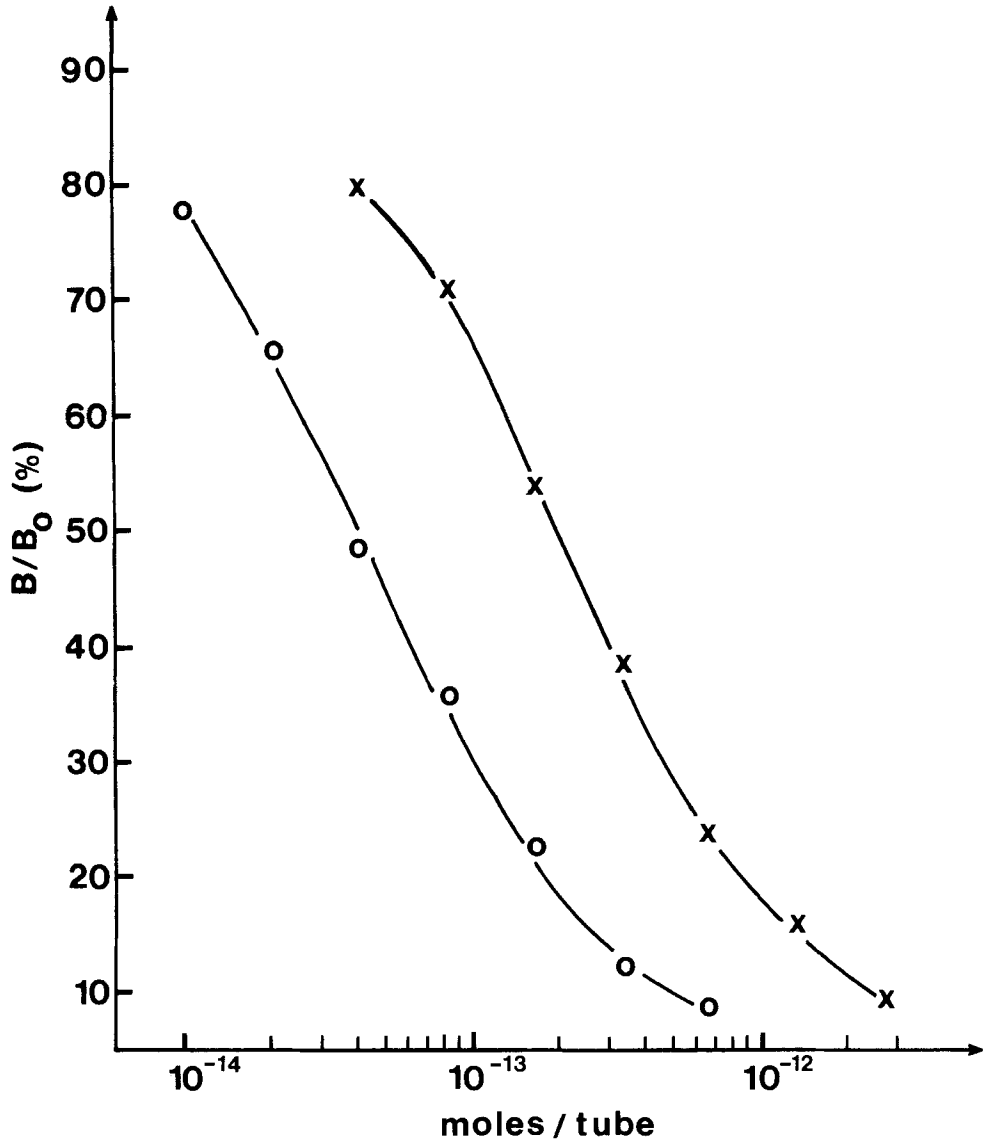


Figure 2

Inhibition of the binding of labelled DAN 2163 with anti DAN 2163 antiserum in the presence of increasing amounts of unlabelled DAN 2163 using ^{3}H-DAN 2163 (X--X) and ^{125}I-DAN 2163 (O--O).

B = Bound radioactivity in présence of competitor

Bo = Bound radioactivity in absence of competitor.

Final dilution of the antiserum yielded 50 % initial binding.

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