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PRELIMINARY COMMUNICATION

PHOTOAFFINITY LABELING OF HUMAN BRAIN DOPAMINE RECEPTORS

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During recent years, a considerable amount of research has been directed towards the characterization and purification of hormone and drug receptors. Neuroleptic drugs are thought to act by blocking brain dopamine receptors; in vitro binding assays using either [^{3}H] haloperidol [1,2] or [^{3}H] spiperone [3] have provided more direct evidence for this interaction. Although [^{3}H] spiperone was also found to label serotonin-S_2 receptors [4], it remains the more appropriate ligand when used with selective displacers since it is capable of labeling dopamine receptors both in vitro and in vivo [5] and even when the receptor has been reduced to a macromolecular form [6]. Dopamine receptors have also been identified in human brain by in vitro binding assays [7].

Photoaffinity labeling has been a valuable tool for the elucidation of the molecular structure of several receptor sites [8,9]. Recently, we developed a photoaffinity compound azapride (4-azido-5-chloro-2-methoxy-N-[1-(phenylmethyl)-4-piperidinyl]benzamide) which is an azide analogue of clebopride, a selective dopamine antagonist [10,11]. The synthesis and the binding characteristics in dog striatum will be described elsewhere [12]. In the present paper, we report on the use of this photoaffinity probe to label dopamine receptors from human brain.

MATERIALS AND METHODS

Drugs and chemicals. Drugs were kindly provided by their companies of origin. [^{3}H] Spiperone (22.9 Ci/mmole) was obtained from N.E.N. (Boston, U.S.A.). All common reagents were obtained from different suppliers and were of the highest purity available.

Tissue preparation. Post-mortem human brains, without any sign of neurological disease, were obtained 3 to 10 hours after death (Cliniques Universitaires Saint-Luc, U.C.L., Brussels). Putamen and caudate nucleus were dissected and immediately put on ice. An MLP-fraction was prepared as previously described [13]. This fraction was diluted with 50 mM Tris-HCl pH 7.7 (buffer A) 1:40 w/v (original wet weight of tissue per volume) and centrifuged for 20 min at 16 000 rpm in a Sorvall RC5B centrifuge (SS-34 rotor). The pellet was washed once with buffer A and again centrifuged. It was then suspended in water (dilution 1:10 w/v) using a glass-teflon homogenizer and stored frozen at -80° C. Before use samples were thawed, homogenized in 50 mM Tris-HCl, pH 7.7 containing 120 mM NaCl (buffer B) and centrifuged for 20 min at 16 000 rpm. The final pellet was suspended in buffer B at a dilution of 1:50 w/v.

Photolabeling. Aliquots of the membrane preparation were incubated with azapride for 15 min at 37° C, with or without addition of other drugs, and then cooled in ice. The samples were photolyzed in quartz test tubes by irradiation with UV-light (long-wavelength, 366 nm) at 4° C and at an average distance of 4 cm using a Camag Universal UV-lamp (Camag, Muttenz, Switzerland) equipped with an 8 W low-pressure mercury tube. During the irradiation, samples were continuously mixed using a multi-axle rotating mixer. Following photolysis, the samples were diluted with buffer A and centrifuged for 15 min at 16 000 rpm. The pellets were washed twice with buffer A by homogenization and centrifugation for 10 min at 16 000 rpm. The final pellets were suspended in buffer B at a dilution of 1:50 w/v and used for binding assays.

[^{3}H] Spiperone binding. Binding assays were performed as previously described [14]. Aliquots of the membrane preparation were incubated for 15 min at 37° C with 1 nM [^{3}H] spiperone, with or without addition of various concentrations of unlabeled drugs and then filtered under suction through Whatman GF/B glass fiber filters. Specific binding was defined as the portion of the total binding which was inhibited by 1 µM domperidone.

RESULTS

Affinity of azapride. Azapride and the parent compound clebopride were tested in [^{3}H] spiperone binding using human striatal membranes. Azapride competed at relatively low concentrations (IC_{50}-value of 266 ± 22 nM, mean ± S.D. of two experiments performed in triplicate). This was about twelve times higher than that for clebopride (IC_{50} = 21 ± 1 nM). After being irradiated for 30 min with long-wavelength UV-light the affinity of azapride was almost unchanged (IC_{50} = 224 ± 65 nM).

Photolabeling of human dopamine receptors. Fig. 1A shows [^{3}H] spiperone binding to human dopamine receptors following preincubation with 10^{-6} M azapride and irradiation for increasing periods of time. [^{3}H] Spiperone binding decreased rapidly during the first two minutes, reaching a minimum after 5 min. In control experiments, without azapride, dopamine receptors were found to retain their binding activity even after longer periods of irradiation. Fig. 1B shows [^{3}H] spiperone binding in membrane fractions preincubated with increasing concentrations of azapride and photolyzed for 10 min. At concentrations between 10^{-8} M and 10^{-5} M of azapride there was an almost linear relationship between [^{3}H] spiperone binding and the azide concentration. At the highest concentration used, more than 90 % of receptors were irreversibly labeled. In the absence of photolysis, azapride was found to dissociate completely from the receptor at all the concentrations used.

Specificity of photolabeling. Fig. 2 shows the competition between different compounds and the photolabel for binding to human dopamine receptors. Azapride was used at a concentration of 10^{-7} M and samples were photolyzed for 10 min. Droperidol and clebopride inhibited at nanomolar concentrations, whereas tetralin [2-(N,N-dipropyl)-amino-5,6-dihydroxytetralin)] [6], a potent dopamine agonist, showed inhibition at slightly higher concentrations. Ketanserin, a potent serotonin antagonist, did not inhibit, even at high concentrations.

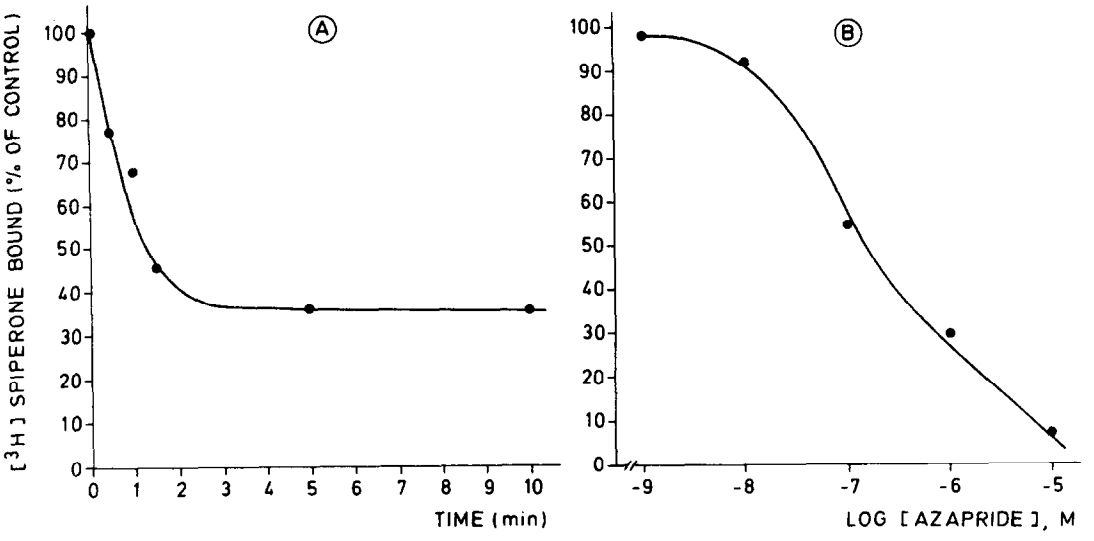

Fig. 1. [^{3}H] Spiperone binding on human striatal membranes after preincubation with azapride and photolysis.
A. Effect of irradiation during increasing periods of time after preincubation with 10^{-6} M azide.
B. Effect of increasing concentrations of azide, during the preincubation which was followed by irradiation for 10 min.

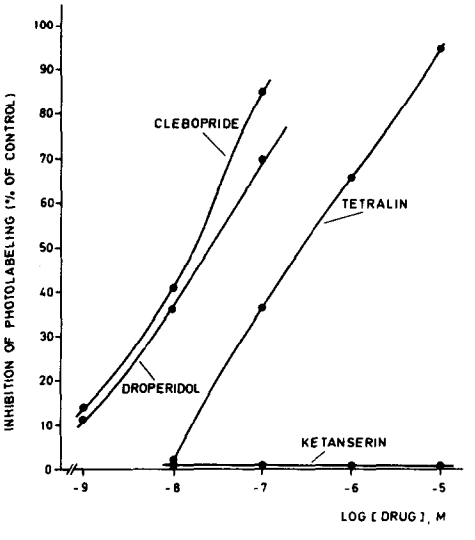

Fig. 2. Inhibition by various compounds of photolabeling with azapride. Striatal membrane fractions were preincubated with 10^{-7} M azapride and increasing concentrations of various drugs. They were subsequently photolyzed for 10 min and assayed for [^{3}H] spiperone binding. The difference between binding to a non-photolyzed and a maximally photolyzed preparation was taken as the 100 % control value. Binding in membranes maximally photolyzed was taken as the 'blank' and was substracted from all results [tetralin = 2-(N,N-dipropyl)-amino-5,6- dihydroxytetralin].

DISCUSSION

Azapride, a selective D_2 receptor antagonist [12] was found to inhibit [^{3}H] spiperone binding in human striatal membranes with a relatively high affinity. Irradiation with UV-light did not affect the affinity of the compound. Following preincubation, azapride was found to irreversibly bind to human dopamine receptors after UV-irradiation. The photoaffinity reaction was dependent on both time of irradiation and azide concentration. Photolabeling with azapride in human striatal membrane preparations was specific for the dopamine receptor; it could be inhibited by dopamine agonists and antagonists, but not by a potent serotonin antagonist.

A problem with photoaffinity labeling is the relatively low degree of labeling, which can be achieved. This problem was not encountered in the present experimental conditions, since azapride labeled up to more than 90 % of receptor sites, when a concentration of 10^{-5} M was used. Moreover, the amount of labeled receptors was found to be nicely related to the azide concentration, the half-maximally effective concentration being 1.3×10^{-7} M. This value correlates nicely with the IC_{50}-value for inhibition by azapride of [^{3}H] spiperone binding to human striatal dopamine receptors ($IC_{50} = 2.6 \times 10^{-7}$ M).

In conclusion, the results of this study show that azapride is a potent photoaffinity probe for human brain dopamine receptors. Radioactively labeled azapride is currently being synthesized. This new radioactive ligand should be of great help in the isolation, purification and further molecular characterization of the human brain dopamine receptor.

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