Biochemical Pharmacology, Vol. 35, No. 19, pp. 3199–3202, 1986.
Printed in Great Britain.

0006–2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

RAPID COMMUNICATION

7-AMINO-8-[^{125}I]-KETANSERIN ([^{125}I]AMIK), A HIGHLY SENSITIVE, SEROTONIN-S_2 RECEPTOR LIGAND

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(Received 25 June 1986; accepted 1 August 1986)

Several [^{3}H] radioligands have been proposed for in vitro biochemical characterization of serotonin-S_2 receptors. Among the most widely used are [^{3}H]spiperone and [^{3}H]ketanserin. Recently, a derivative of ketanserin, [^{3}H]7-aminoketanserin has been introduced as a high affinity selective serotonin-S_2 receptor ligand with a very low non-specific binding [1]. A major drawback of all these ligands, however, is the low specific radioactivity especially if one wants to study tissues with low receptor contents. [^{125}I]-Labelled compounds have much greater sensitivity than tritiated probes. The available iodinated serotonin-S_2 receptor ligands [^{125}I]LSD [2] and [^{125}I]MIL (a methylated derivative of [^{125}I]LSD) [3] show, however, the major disadvantage of interacting with other serotonin binding sites at nanomolar ligand concentrations [4]. It therefore seemed desirable to develop a [^{125}I]-labelled compound, based on a selective ligand for the serotonin-S_2 receptor subtype, such as 7-aminoketanserin.

Materials and methods

Drugs and chemicals. 7-Amino-8-iodo-ketanserin (7-amino-3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl]-8-iodo-2,4-(1H,3H)-quinazolinedione) (AMIK) was synthesized from its parent compound 7-aminoketanserin and sodium iodide using the chloramine-T method. The synthesis and complete characterization of the compound will be described elsewhere. 7-Amino-8-[^{125}I]-ketanserin ([^{125}I]AMIK) (~ 2175 Ci/mmol) was custom synthesized and HPLC-purified by Amersham (Amersham, U.K.). [^{3}H]Ketanserin (15 Ci/mmol) and [^{3}H]haloperidol (22 Ci/mmol) were obtained from Janssen Life Sciences Products (Beerse, Belgium); [^{3}H]WB-4101 (20.7 Ci/mmol), [^{3}H]clonidine (66.8 Ci/mmol) and [^{3}H]pyrilamine (26.7 Ci/mmol) from New England Nuclear (Boston, U.S.A.) and [^{3}H]serotonin (14 Ci/mmol) from Amersham (Amersham, U.K.). All drugs were kindly provided by their original manufacturers.

Receptor assays. Determination of K_i-values in different receptor systems was performed using the following radioligands and tissues according to published methods [5, 6, 7]: serotonin-S_2, [^{3}H]ketanserin, rat frontal cortex; serotonin-S_1, [^{3}H]serotonin, rat hippocampus; histamine-H_1, [^{3}H]pyrilamine, guinea-pig cerebellum; dopamine-D_2, [^{3}H]haloperidol, rat striatum; α_1-adrenergic, [^{3}H]WB-4101, rat forebrain; α_2-adrenergic, [^{3}H]clonidine, rat total cortex.

Binding of [^{125}I]AMIK. Female Wistar rats were killed by decapitation and their brains were removed from the skull. Frontal cortex was rapidly dissected and immediately homogenized in 0.25 M sucrose. A total mitochondrial plus microsomal (M+L+P) fraction was prepared by differential centrifugation as previously described [6]. The final fraction was suspended in 50 mM Tris-HCl, pH 7.4, at a dilution of 1:500 (w/v) (original wet weigh of tissue per volume). Aliquots of this final preparation were incubated with [^{125}I]AMIK and other drugs or their solvent at 37° C for 15 min. Bound and free radioligand were separated by filtration over Whatman GF/B glass fibre filters. Filters were counted for radioactivity in Insta Gel II (Packard) using a Packard Tri-Carb 460 liquid scintillation counter equipped with a DPM-calculation program.

Results and discussion

The binding characteristics of AMIK in a variety of receptor systems were tested. From the obtained IC_{50}-values, K_i-values were calculated using the equation of Cheng and Prusoff [8]. These values are shown in Table 1. Apart from its high affinity for serotonin-S_2 receptors, AMIK also showed binding to histamine-H_1 receptors at nanomolar concentrations. The affinity of AMIK for H_1-receptors was nine times lower than for S_2-receptors. This corresponds to the histaminergic affinities, previously reported for ketanserin and its amino- and azido-derivatives [9]. The affinity of AMIK for α_1-adrenergic receptors was eighteen times lower than for S_2-receptors; the compound was only weakly or not active in the binding to serotonin-S_1, dopamine-D_2 and α_2-adrenergic receptors.

TABLE 1

Binding characteristics of AMIK. The binding to different receptor systems was measured as described in the methods section. K_i-values were calculated using the equation of Cheng and Prusoff [8].

Receptor system	K_i (nM)	$\frac{K_i}{K_i \text{ serotonin-}S_2}$
Serotonin-S_2	0.37	1
Serotonin-S_1	2284	6173
Histamine-H_1	3.42	9.2
α_1-Adrenergic	6.92	18.7
Dopamine-D_2	223	603
α_2-Adrenergic	> 1000	> 1000

Binding of [^{125}I]AMIK to a membrane preparation from rat frontal cortex was reversible and saturable. Scatchard analysis of the saturation plots were linear with a dissociation constant K_D = 0.14 ± 0.01 nM (mean ± S.D.). Using a final tissue concentration of 1:500 (original wet weight of tissue per volume) and 0.1 nM [^{125}I]AMIK, non-displaceable binding (determined using an excess of methysergide) was typically lower than 25 % of total binding.

Several compounds were tested for their potency to inhibit [^{125}I]AMIK binding to a membrane preparation from rat frontal cortex. The derived IC_{50}-values are shown in Table 2. Binding was potently inhibited by serotonin antagonists (ketanserin, pipamperone and methysergide), whereas the other antagonists, pyrilamine (histamine-H_1), domperidone (dopamine-D_2) and prazosin (α_1-adrenergic) were only weakly or not active. Serotonin agonists (serotonin and bufotenine) were active at micromolar concentrations.

TABLE 2

Competition of various compounds in the binding of [^{125}I]AMIK to a (M+L+P) (total mitochondrial and microsomal) fraction of rat frontal cortex. The assay was performed as described in the methods section, using 0.1 nM final radioligand concentration. Values are means ± S.D. of two or three independent experiments, each performed in duplicate.

Compound	-log IC_{50} (M)
Pipamperone	8.77 ± 0.06
Methysergide	8.56 ± 0.12
Ketanserin	8.44 ± 0.17
Domperidone	6.67 ± 0.11
Pyrilamine	5.85 ± 0.00
Prazosin	5.66 ± 0.19
Bufotenine	6.32 ± 0.15
Serotonin	5.79 ± 0.02

The foregoing results demonstrate that [^{125}I]AMIK is a very potent, specific serotonin-S_2 receptor ligand with a very good ratio of specific to non-specific binding. This makes it the ligand of choice to study serotonin-S_2 receptors in those tissues, having a low receptor content. Apart from its high affinity for serotonin-S_2 receptors, the compound also shows nanomolar affinity for histamine-H_1 receptors. This dual affinity is not a disadvantage if selective displacers (such as methysergide) are used to define specific serotonin-S_2 receptor binding. Eventually, the new compound may also be useful to study histamine-H_1 receptors.

Acknowledgements. We thank L. Geentjens for secretarial work. Part of this work was supported by a grant from I.W.O.N.L.

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