INTERACTION BETWEEN THE ANXIOLYTIC BUSPIRONE WITH SEROTONIN AND OTHER SYNAPTIC RECEPTORS OF THE HUMAN BRAIN

A. Ya. Korneev, M. I. Faktor, and Chan Tkhi Bin' An

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Buspirone[8-(4-)4-(2-pyrimidinyl)-1-piperazinyl(butyl)-8-azaspiro(4,5)-decane-7,9-dione] exhibits anxiolytic properties similar to those of diazepam, but does not possess the sedative and anticonvulsant activity of the latter, and according to the results of a number of investigations, it does not interact with the GABA-benzodiazepine receptor complex [8, 10]. The study of the molecular mechanisms of action of buspirone by the radioreceptor method revealed relatively high affinity for dopamine D_2 receptors and serotonin C_1-receptors in bovine and rat brain [6, 8]. There is some evidence that dopamine D_2-receptors are not involved in the realization of the anxiolytic effect of buspirone [10, 11].

For the reasons given above it was decided to study interaction of buspirone with various components of the serotoninergic system of the human brain, and also with specific binding sites for tryptamine, imipramine, flunitrazepam, and strychnine.

EXPERIMENTAL METHOD

Human brains obtained 10-12 h after death from nine patients (five men and four women) aged 60-84 years, dying from causes unconnected with brain pathology, were used. Isolated tissue of the cortex and hippocampus was kept at −20°C for not more than 2 weeks, and after thawing, it was homogenized (Polytron, 30 sec, maximal speed) in 40 volumes of 50 mM Tris-HCl buffer (pH 7.4, 20°C), cooled to 2°C, and centrifuged for 15 min at 20,000g and 4°C. Rehomogenization of the residue in fresh buffer and centrifugation were repeated 3 more times under the same conditions, after which the residues were frozen overnight. The thawed residues were washed under similar conditions once more and suspended in incubation buffer in a final concentration of 20 mg/ml, calculated relative to the original weight of tissue.

Specific binding of ^{3}H-LSD and ^{125}I-LSD was determined by the methods in [2, 5] with minimal modifications. Samples with a volume of 1 ml containing a suspension of membranes in a final concentration of 10 mg/ml in 50 mM Tris-HCl (pH 7.4, 20°C), the radioactive ligand in a concentration of 2 nM for ^{3}H-LSD and 10 nM for ^{125}I-LSD, as well as the added test substances, were incubated at 37°C for 30 min, after which they were filtered quickly in vacuo through GF/B filters (25 mm, Whatman, England) and washed with 3 aliquots (each of 5 ml) of ice-cold incubation buffer.

Specific binding of ^{3}H-serotonin was determined by the method in [5] with modifications. Samples of 1 ml containing a suspension of tissue in a final concentration of 10 mg/ml in buffer − 50 mM Tris-HCl, 1 mM ascorbic acid, 1 μM pargyline (pH 7.7, 20°C), 3 mM ^{3}H-serotonin − and the added test substances were incubated at 4°C for 40 min, then filtered in vacuo as described above.

Nonspecific binding of the labeled ligands was determined in the presence of 100 μM serotonin. Specific binding of ^{3}H-tryptamine, ^{3}H-imipramine, ^{3}H-flunitrazepam, and ^{3}H-strychnine in concentrations of 5, 5, 1, and 5 nM, was determined by the methods in [1, 7, 4, 12].

The degree of binding was determined by measuring radioactivity retained on the filter. Total binding was determined in three or four parallel tests, nonspecific binding in three parallel tests, and specific binding was obtained by subtracting nonspecific from total. To

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TABLE 1. Parameters of Specific Binding of Serotoninergic Ligands with Human Brain Cell Membranes (M ± m)

Parameter	^{3}H-serotonin (3 nM)		^{3}H-LSD (2 nM)		^{125}I-LSD (110 nM)	
	cortex	hippo-campus	cortex	hippo-campus	cortex	hippo-campus
SB, pmoles/g tissue	1,1±0,2	1,0±0,2	6,8±0,8	3,6±0,4	91,6±28,0	54,7±16,1
SB/TB, %	50,5	46,5	80,0	72,2	63,8	54,0
$\frac{\text{SB (hippocampus)}}{\text{SB (cortex)}} \cdot 100\%$	90		53		60	

Note: SB) Specific binding, TB) total binding.

TABLE 2. Inhibition of Specific Binding of Serotoninergic Ligands with a Membrane Preparation from the Human Hippocampus and Cerebral Cortex by Buspirone and Mj 138-05 (M ± m)

Inhibitor	IC_{50} for specific binding, μM		
	^{3}H-serotonin (3 nM)	^{3}H-LSD (2 nM)	^{125}I-LSD (10 nM)
Hippocampus			
Serotonin	0,005±0,003	0,14±0,08	4,0±0,8
Buspirone	3,8±0,4	2,3±0,9	2,5±0,5
Mj 138-05	21,0±7,0	6,1±1,4	6,6±1,9
Spiroperidol	4,4±1,2	0,08±0,05	0,20±0,08
Cortex			
Serotonin	0,008±0,005	2,2±0,7	6,2±0,9
Buspirone	>30	8,2±0,8	3,7±0,7
Mj 138-05	>50	23,2±4,3	7,2±0,6
Spiroperidol	4,1±1,5	0,1±0,04	0,03±0,01

Note: Mean results of 6-9 experiments.

determine IC_{50} (the concentration giving half the maximal inhibition) five concentrations of the test substance were used within the range 10 nM-100 μM, each one in three parallel determinations.

The following reagents were used: ^{3}H-LSD with specific activity of 16 Ci/mmole; ^{125}I-LSD – 2000 Ci/mmole; ^{3}H-serotonin – 16.9 Ci/mmole; ^{3}H-tryptamine – 3.5 Ci/mmole; ^{3}H-strychnine – 15 Ci/mmole; ^{3}H-flunitrazepam – 72 Ci/mmole; and ^{3}H-imipramine – 23 Ci/mmole were from Amersham International (England); serotonin was from Sigma (USA); and spiroperidol from Janssen (Belgium). Buspirone and compound Mj 138-05 were obtained from the Bristol-Myers Company (USA).

EXPERIMENTAL RESULTS

Specific binding of labeled tryptamine, strychnine, flunitrazepam, and imipramine in the human cerebral cortex amounted to 72, 75, 95, and 60% respectively of total binding. Buspirone and Mj 138-05, in concentrations up to 100 μM, did not inhibit specific binding of the above-mentioned ligands with cell membranes of the hippocampus and cerebral cortex. As regards glycine and benzodiazepine receptors these data agree with those in the literature [8]. For instance, buspirone and Mj 138-05 do not interact with serotonin reuptake sites revealed by the use of imipramine, or with specific binding sites of tryptamine, whose functional role is not yet clear.

Parameters of binding of labeled serotonin and LSD with serotonin receptors of the human cerebral cortex and hippocampus are given in Table 1. Clearly serotonin receptors revealed by the aid of serotonin quantitatively, are represented about equally in the hippocampus and cortex, whereas serotonin receptors revealed by the use of ^{3}H-LSD and ^{125}I-LSD predominate in the cortex. Data on inhibition of specific binding of the three serotoninergic radioligands used by serotonin and spiroperidol (Table 2) are in good agreement with data in the literature [3, 5]. According to existing ideas serotonin C_1-receptors, characterized by high affinity for serotonin (nanomoles) and by relatively low affinity (micromoles) for

spiroperidol, which predominate in the cerebral cortex, exhibit high affinity for serotonin antagonists such as spiroperidol and ketanserin (nanomoles) and much lower affinity for serotonin (micromoles) and its antagonists [3, 5]. It is considered that ^{3}H-LSD exhibits equal affinity for serotonin C_1- and C_2-receptors, whereas ^{125}I-LSD is a selective ligand for C_2-receptors [2, 5]. It can be concluded from the views described above and data on activity of buspirone and its analog Mj 138-05 given in Table 2 that both compounds exhibit affinity similar to that of serotonin (IC_{50} in 2-6 μM diapazone) for serotonin C_2-receptors, discovered both in the hippocampus and in the cortex with the aid of ^{125}I-LSD. This conclusion in the case of buspirone is in good agreement with data obtained by other workers [6, 8, 10]. Meanwhile Skolnick and Paul [10] report absence of affinity of Mj 138-05 for serotonin C_2-receptors discovered in rat brain with the aid of ^{3}H-ketanserin. The reason for this disagreement may be differences in the properties of C_2-receptors in the human and rat brain, or differences in populations of serotonin receptors discovered by the aid of ketanserin and I-LSD. Evidence in support of the latter hypothesis is given by the much lower affinity of Mj 138-05 than that of buspirone and serotonin for serotonin receptors revealed with the aid of ^{3}H-LSD in the cortex (Table 2).

Data showing high affinity of buspirone for a subtype of serotonin C_1-receptors, namely C_{1A}-receptors, revealed by the aid of selective ligands ^{3}H-DRAT and ^{3}H-TVXQ 7821, have recently been published. The value of IC_{50} for buspirone in relation to specific binding of ^{3}H-DRAT with serotonin C_{1A}-receptors in bovine hippocampus is 20 nM, whereas its IC_{50} for C_{1B}-receptors (another subtype of C_1-receptors) is 20-30 μM for bovine brain [6]. Since serotonin exhibits equal affinity for both C_{1A}- and C_{1B}-receptors (nanomoles), the more numerous the C_{1B}-receptors in a given tissue, the higher the value of IC_{50} of buspirone for specific serotonin binding will be. Consequently, the presence of C_{1B}-receptors could be the reason for the relatively low affinity of buspirone for C_1-receptors revealed with the aid of serotonin in the human hippocampus (Table 2). At the same time, according to data published by Hoyer et al. [3], there are no serotonin C_{1B}-receptors in the human brain. The results of the present investigation may therefore be evidence either of considerable differences in the properties of serotonin C_{1A}-receptors in the human brain compared with the rat and bovine brain (for example, of their heterogeneity with respect to affinity for buspirone), or of marked age changes in these receptors, leading to a decrease of their affinity for buspirone. The possibility of such age changes in the properties of serotonin receptors is confirmed by data in the literature [9].

To sum up, it can be concluded that the anxiolytic buspirone interacts in micromolar concentrations with serotonin C_1- and C_2-receptors of the human brain.

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