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Binding Potency of 6-Nitroquipazine Analogues for the 5-Hydroxytryptamine Reuptake Complex

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Abstract—The in-vitro inhibition constants (K_i) of nine structural analogues of the potent 5-hydroxytryptamine (5-HT)-uptake inhibitor, 6-nitroquipazine, were determined to assess the structure-affinity relationship of these derivatives. The goal of these studies was to determine those positions on 6-nitroquipazine that could be derivatized without significantly decreasing the affinity of the drug for the binding site, so that radiolabels such as ^{123}I, ^{76}Br or ^{18}F might be appended for in-vivo imaging studies of the 5-HT reuptake system. Using bromine as a steric probe, the rank order of potency of bromine-substituted 6-nitroquipazine analogues for inhibiting the binding of [^{3}H]paroxetine to the 5-HT reuptake binding site was: 8-<3-<7-<4-<5-bromo. The in-vitro equipotent molar ratio (EPMR, K_i (analogue)/K_i(6-nitroquipazine)) of the 5-bromo analogue was 0·57, indicating that this analogue had greater affinity for the 5-HT reuptake complex than 6-nitroquipazine. Derivatization at the 5-position with fluorine and iodine also resulted in potent compounds with EPMR values of 1·1 and 0·83, respectively. Substitution of quipazine with bromo, cyano, and formyl groups at the 6-position produced less potent compounds than the 6-nitro group. Based upon the high affinities of the 5-bromo-, 5-fluoro- and 5-iodo-6-nitroquipazines for the 5-HT reuptake complex, these compounds are candidates for radiolabelling for in-vivo studies of the 5-HT reuptake site.

Interest in the development of radioligands for in-vivo neuroreceptor studies using single photon emission computed tomography (SPECT) and positron emission tomography (PET) has increased in recent years (Phelps et al 1986; Fowler et al 1990; Kung 1990; Maziere & Maziere 1991). Most of the in-vivo receptor imaging studies to date have utilized radioligands which bind to postsynaptic sites, but efforts have recently been directed towards the development of presynaptic radioligands which bind reuptake complexes (Neumeyer et al 1991; Shaya et al 1992; Frost et al 1993; Kilbourn et al 1993; Suehiro et al 1993). In-vitro and ex-vivo investigations have indicated that the regional cerebral concentrations of 5-hydroxytryptamine (5-HT) uptake sites are proportional to regional 5-HT-ergic innervation (De Souza & Kuyatt 1987; Cortes et al 1988). In-vivo studies of the presynaptic 5-HT reuptake complex are of interest to determine changes in this system with depression (Perry et al 1983; Gross-Isseroff et al 1989), ageing (Veith & Raskind 1988), drug abuse (Battaglia et al 1987), and Parkinson's and Alzheimer's diseases (Raisman et al 1986; D'Amato et al 1987; Chinaglia et al 1993). In an effort to develop useful radioligands for SPECT and PET studies of the 5-HT reuptake complex, analogues of the selective and potent 5-HT reuptake inhibitor 6-nitroquipazine (6-nitro-2-piperazinylquinoline, Fig. 1) were synthesized, and their in-vitro inhibition constants (K_i) were determined in competition studies utilizing [^{3}H]paroxetine binding to rat cortical membranes.

6-Nitroquipazine selectively binds to the 5-HT transporter complex located on presynaptic 5-HT neurons of the central nervous system (Hashimoto & Goromaru 1990a, 1991) and has one of the highest affinities for the 5-HT uptake site of any compound reported to date. In addition, 6-nitroquipazine has low affinity for non-5-HT-ergic transporter systems and other pre- and postsynaptic receptor sites. In-vitro studies of [^{3}H]6-nitroquipazine binding to cerebral tissue indicated that this ligand bound selectively to regions of rat brain known to contain high densities of the 5-HT reuptake complex (Hashimoto & Goromaru 1990a). In-vivo studies of [^{3}H]6-nitroquipazine in mice have demonstrated that the distribution of specific binding paralleled the distribution of 5-HT uptake sites in rodent brain (Hashimoto & Goromaru 1990b). The specific in-vivo binding of [^{3}H]6-nitroquipazine was selectively blocked by pretreatment of the animals with 5-HT uptake inhibitors, and pretreatment of rats with the 5-HT-ergic neurotoxin 3,4-methylenedioxymethamphetamine (MDMA) led to a reduction in specific [^{3}H]6-nitroquipazine binding in-vivo (Hashimoto & Goromaru 1990c). These in-vitro and in-vivo data indicate that single photon- or positron-labelled analogues of 6-nitroquipazine would probably be useful as SPECT or PET radioligands for studying 5-HT neurons in living human brain. SPECT studies using ^{123}I radiolabel (half-life 13 h) are attractive from both radiosynthetic and practical aspects resulting from the relatively long half-life and commercial availability of this radionuclide. A long half-life radiolabel may be necessary so that relatively low

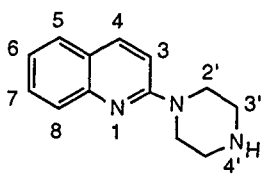

FIG. 1. Structure of quipazine showing the ring systems and position numbering schemes.

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in-vivo non-specific binding clearance can be effected (Hashimoto & Goromaru 1990b; Scheffel & Ricaurte 1990). PET imaging studies of a positron-labelled 6-nitroquipazine analogue utilizing either ^{76}Br (half-life 16 h), ^{18}F (half-life 110 min) or ^{11}C (half-life 20 min) provide a wide range of half-lives and radiosynthetic possibilities.

6-Nitroquipazine does not contain iodine, bromine, or fluorine, and the incorporation of a ^{11}C radiolabel into the carbon framework is not easily affected (Fig. 1). Therefore, it was necessary to identify those positions on the molecule which could be derivatized without significantly decreasing the binding affinity of the analogue for the 5-HT reuptake site. The in-vitro and in-vivo binding characteristics of ^{125}I-labelled 5-iodo-6-nitroquipazine (Biegon et al 1993; Mathis et al 1993), as well as preliminary in-vivo SPECT imaging studies of 5-[^{123}I]iodo-6-nitroquipazine in monkeys (Jagust et al 1993), have recently been described. The effects of derivatization on the binding potency of 6-nitroquipazine at six different positions are reported here to characterize the structure-affinity relationship of these analogues and to assess these sites for derivatization and radiolabelling.

Materials and Methods

In-vitro binding studies

Receptor binding assays were performed according to the methods of Habert et al (1985). In brief, cerebral cortex from adult rat brains (Pel-Freeze Biologicals, Inc., Rogers, AK) was dissected and homogenized in 20 vol 50 mM Tris-HCl buffer (pH 7·4 at 25°C) using a Brinkmann Polytron tissue homogenizer and subsequently centrifuged (49 000 *g*) for 10 min. The supernatant was discarded, and the pellet was resuspended in the same volume of Tris-HCl buffer and incubated at 37°C for 10 min before a second centrifugation at 49 000 *g* for 10 min. The final pellet was resuspended at a concentration of approximately 1 mg wet wt mL^{-1} in 50 mM Tris-HCl buffer (pH 7·4) containing 120 mM NaCl and 5 mM KCl. The tissue suspension was immediately used in the binding assays.

Binding to the 5-HT reuptake complex was determined using 0·1 mL pH 7·4 Tris-HCl buffered [^{3}H]paroxetine (sp. act. 20·5 Ci mmol^{-1}, final binding assay concentrations 0·2–0·3 nM) as the radioligand, 0·1 mL buffered competing quipazine analogue (added in increasing concentrations from 10^{-10} to 10^{-5} M), and 0·8 mL tissue suspension. Non-specific binding was determined by adding 0·1 mL buffered 10 μM paroxetine to 0·1 mL [^{3}H]paroxetine stock solution and 0·8 mL tissue suspension. Total binding was assessed by adding 0·1 mL [^{3}H]paroxetine stock solution of 0·1 mL Tris-HCl buffer solution and 0·8 mL of the tissue suspension. Competition incubations were performed at 25°C for 60 min, and 5 mL ice-cold 50 mM Tris-HCl buffer (pH 7·7 at 4°C) was then added to the assays. The solutions were rapidly filtered under vacuum through Schleicher and Schuell (Keene, NH) #32 glass fibre filters using a Brandell cell harvester. The filters were washed twice with 5 mL 50 mM Tris-HCl buffer (at 4°C) and air-dried. The filters were transferred to scintillation vials, and 5 mL water was added. The vials were shaken for 60 min at room temperature (21°C), 10 mL Ultimagold liquid scintillation fluid (Packard) was added, and the levels of radioactivity were determined on a Packard Model 2500TR liquid scintillation counter at approximately 41% efficiency. Data from the competition assays were plotted as percent of total specific binding vs log molar concentration of unlabelled (competing) drug. The IC50 value (inhibitor concentration of 50% [^{3}H]paroxetine binding inhibition) was determined from a Hill plot of six or more data points. The inhibition binding constant (K_i) was calculated according to the relationship: $K_i = IC50/(1 + [L]/K_D)$, where [L] is the concentration of unbound [^{3}H]paroxetine and K_D is 0·15 nM (Habert et al 1985). All determinations were performed in triplicate, and the mean values ± s.e.m. are reported.

Drugs

[^{3}H]Paroxetine was purchased from New England Nuclear (Boston, MA). Paroxetine hydrochloride was a gift from Beecham Pharmaceuticals (Betchworth, Surrey, UK), and quipazine was purchased from Research Biochemicals Incorporated (Natick, MA). The structural analogues of quipazine and 6-nitroquipazine (compounds 2–14, see Table 1) were synthesized at Lawrence Berkeley Laboratory and the University of Pittsburgh, and the spectroscopic properties (^{1}H NMR and IR), elemental analyses, and chromatographic profiles (TLC and HPLC) were consistent with the assigned structures. The equipotent molar ratios (EPMR) of the analogues (i.e. the ratio of the K_i of the analogue to the K_i of 6-nitroquipazine (compound 2)), indicate the relative potency of the analogues in competing for the 5-HT reuptake site compared with 6-nitroquipazine.

Results and Discussion

A representative in-vitro competitive binding plot of several brominated 6-nitroquipazine analogues is shown in Fig. 2, and the in-vitro binding results are presented in Table 1. Nitration of quipazine (compound 1) at the 6-position resulted in a derivative (2) which was a potent inhibitor of

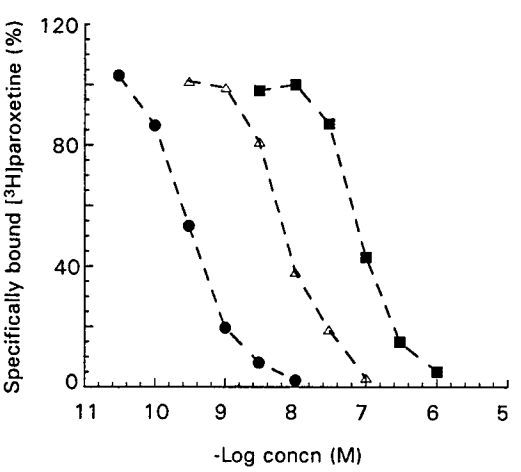
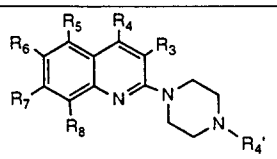


FIG. 2. Inhibition of the binding of [^{3}H]paroxetine to rat cortical membranes with various brominated 6-nitroquipazine analogues. Results are expressed as a percentage of specific binding defined by 10 μM paroxetine, and the data are from typical experiments performed in triplicate. ● 5-Bromo-6-nitroquipazine (compound 8), △ 7-bromo-6-nitroquipazine (compound 9), ■ 8-bromo-6-nitroquipazine (compound 10).

Table 1. Sites of substitution, in-vitro inhibition binding constants (K_i)[a], and equipotent molar ratios (EPMR)[b] of quipazine and quipazine analogues.



Compound	R_3	R_4	R_5	R_6	R_7	R_8	$R_{4'}$	K_i(nM)	EPMR
1	H	H	H	H	H	H	H	68 ± 18	300
2	H	H	H	NO_2	H	H	H	0·23 ± 0·06	1·0
3	H	H	H	Br	H	H	H	0·69 ± 0·35	3·0
4	H	H	H	CN	H	H	H	5·6 ± 2·8	24
5	H	H	H	CHO	H	H	H	27 ± 14	120
6	Br	H	H	NO_2	H	H	H	9·6 ± 2·6	42
7	H	Br	H	NO_2	H	H	H	0·40 ± 0·25	1·7
8	H	H	Br	NO_2	H	H	H	0·13 ± 0·02	0·57
9	H	H	H	NO_2	Br	H	H	4·1 ± 1·5	18
10	H	H	H	NO_2	H	Br	H	18 ± 3	78
11	H	H	H	NO_2	H	H	CH_3	52 ± 9	230
12	H	H	H	NO_2	H	H	$(CH_2)_2F$	120 ± 30	520
13	H	H	I	NO_2	H	H	H	0·19 ± 0·09	0·83
14	H	H	F	NO_2	H	H	H	0·25 ± 0·15	1·1

[a]Rat cortical membranes were labelled with [^{3}H]paroxetine. The IC50, from which the K_i was calculated, was determined from a Hill plot of six data points in triplicate. The K_i data represent the mean ± s.e.m. of three or more individual determinations performed on separate days. [b]The EPMR of the analogues is the ratio of the K_i of the quipazine analogue to the K_i of 6-nitroquipazine.

[^{3}H]paroxetine binding to the 5-HT reuptake complex. In addition, bromination at the 6-position likewise resulted in a potent inhibitor of [^{3}H]paroxetine binding. These results are in close agreement with those previously reported by Hashimoto & Goromaru (1990a, 1992). Derivatization at the 6-position with other electron withdrawing groups, such as cyano and formyl (compounds 3, 4), yielded less potent 5-HT reuptake complex ligands. Additional steric probing of the 3-, 4-, 5-, 7-, and 8-positions in the quinoline ring of 6-nitroquipazine with bromine (compounds 6–10) indicated that the 4- and 5-positions were the most tolerant to substitution. In fact, the 5-bromo analogue (8) was more potent than the parent compound. The 5-iodo and 5-fluoro (compounds 13, 14) were also extremely potent, indicating a relatively high degree of bulk tolerance at this position. However, derivatization of the 4′-nitrogen of the piperazine ring with methyl and β-fluoroethyl groups resulted in a considerable loss in binding potency. Thus, it is unlikely that the 4′-[^{11}C]methyl and 4′-(β-[^{18}F]fluoroethyl) analogues will be useful radioligands.

Conclusions

Studies of the effects of bromine derivatization at six different positions in 6-nitroquipazine identified the 5-position as the best site for halogen substitution. The rank order of potency of the various brominated 6-nitroquipazine analogues for inhibiting the binding of [^{3}H]paroxetine to the 5-HT transporter was: 8-<3-<7-<4-<5-bromo. The 4′-methyl and 4′-(β-fluoroethyl) analogues of 6-nitroquipazine were much less potent than the parent compound in inhibiting [^{3}H]paroxetine binding.

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