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Indorenate modifies a_1-adrenergic and benzodiazepine receptor binding in the rat brain: an autoradiography study

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Abstract

Indorenate (5-methoxytryptamine-β-methylcarboxylate) is a 5-HT_{1A} receptor agonist that produces antihypertensive, anxiolytic, antidepressant and anticonvulsant effects. However, there is evidence suggesting that these effects could involve the activation of benzodiazepine (BZD) receptors but not the activation of a_1-adrenergic receptors. The goal of this study was to analyse the effect of indorenate on a_1-adrenergic and BZD receptor binding in specific rat brain areas by using in-vitro autoradiography. Coronal brain sections from male Wistar rats were used for labelling 5-HT_{1A} (^{3}H-8-OH-DPAT, 2 nM), a_1-adrenergic (^{3}H-prazosin, 2 nM) and BZD (^{3}H-flunitrazepam, 2 nM) receptor binding in the presence or absence of indorenate (1 μM). Indorenate totally displaced ^{3}H-8-OH-DPAT binding in all the brain areas evaluated. It decreased ^{3}H-prazosin binding just in the frontal (30%) and sensorimotor (32%) cortices and in the thalamus (21%). Additionally, indorenate diminished ^{3}H-flunitrazepam binding only in the cingulate (16%) and piriform (18%) cortices as well as in the dorsal raphe nucleus (18%). These results confirm that indorenate is a 5-HT_{1A} ligand and suggest the possible participation of a_1-adrenergic and BZD receptors in its pharmacological properties.

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Introduction

Indorenate (5-methoxytryptamine-β-methylcarboxylate) is a tryptamine analogue with antihypertensive (Hong 1981; Safdy et al 1982), anxiolytic (Fernández-Guasti & López-Rubalcava 1990, 1998), antidepressant (Martínez-Mota et al 2002) and anticonvulsant effects (López-Meraz et al 2005). It produces discriminative stimulus over an operant response as well as an anorectic-like effect through the stimulation of 5-HT_{1A}, 5-HT_{1C} and 5-$HT_{2A/2C}$ receptors (Sánchez & Velázquez-Martínez 2001). In-vitro experiments have shown that indorenate inhibits, in nanomolar concentrations, ^{3}H-serotonin (5-HT) and ^{3}H-ipsapirone binding in hippocampal membranes (Dompert et al 1985) as well as ^{3}H-8-OH-DPAT binding ($K_d = 16$ nM) in cortical membranes (Hoyer et al 1985). With less affinity, indorenate reduces the binding of ^{3}H-mesulergine ($K_d = 316$ nM) and ^{3}H-iodocyanopindolol ($K_d = 3981$ nM) in cortical membranes (Hoyer et al 1985). Altogether, these results suggest that indorenate has high affinity for 5-HT_{1A} receptors and binds with less affinity to 5-HT_{2C} and 5-HT_{1B} receptors.

A functional interaction between brain noradrenergic and serotonergic systems (Mongeau et al 1997; Bortolozzi & Artigas 2003) has been reported. Additionally, data suggest that some pharmacological effects related to activation of 5-HT_{1A} receptors involve the participation of noradrenergic receptors. Some 5-HT_{1A} receptor agonists, such as buspirone and 8-OH-DPAT, act also on vascular a_1-adrenergic receptors (Castillo et al 1993; Osei-Owusu & Scrogin 2004). However, such an interaction has not been reported with indorenate.

A possible interaction between the GABA/BZD (gamma-aminobutyric acid/benzodiazepine) and the 5-HT systems has been suggested by numerous studies. Buspirone, a partial 5-HT_{1A} receptor agonist, enhances ^{3}H-flunitrazepam binding in-vivo in some brain areas (Oakley & Jones 1983). 8-OH-DPAT, a 5-HT_{1A} receptor full agonist, augments ^{3}H-flunitrazepam binding affinity in rat cortical membranes (Sölderpalm et al 1997). Additionally, the anxiolytic effects of 8-OH-DPAT and buspirone, as well as the 5-HT_{1A} receptor antagonists WAY100635, MP 349 and MM 77, are blocked by the BZD receptor antagonist flumazenil

(López-Rubalcava et al 1992; Fernandez-Guasti & López-Rubalcava et al 1998; Wesolowska et al 2003). The anxiolytic effect of indorenate can be blocked by flumazenil as well (López-Rubalcava et al 1992). Altogether, these studies suggest that the $GABA_A$/BZD complex may be indirectly involved in the anxiolytic effects of 5-HT_{1A} receptor ligands.

This study was aimed at investigating whether indorenate is able to modify a_1-adrenergic and BZD receptors binding in the rat brain by autoradiography in-vitro. An autoradiography procedure was preferred to membrane binding experiments because it allows a precise spatial analysis of specific brain structures and subfield. Our results show indorenate is capable of decreasing ^{3}H-prazosin and ^{3}H-flunitrazepam binding in specific brain areas.

Materials and Methods

Animals

Male Wistar rats (n = 8), initially weighing 250–300 g, housed at 22°C and maintained on a 12-h light–dark cycle were used. The rats had free access to food and water. Procedures involving animal care were conducted in agreement with the Mexican Official Norm (Norma Oficial Mexicana, NOM-062-ZOO-1999) and the Ethical Committee of the Centro de Investigación y de Estudios Avanzados (Project 222/04).

Rats were killed by decapitation and brains were quickly removed, frozen in pulverized dry ice and stored at −70°C for subsequent autoradiography experiments.

Drugs

The following drugs were used: indorenate (5-methoxytryptamine-β-methylcarboxylate HCl; Miles-Laboratories and Cinvestav, Mexico), ^{3}H-8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin; specific activity = 135 Ci mmol^{-1}; New England Nuclear), ^{3}H-prazosin (specific activity = 77.2 Ci mmol^{-1}; Amersham Pharmacia Biotech) and ^{3}H-flunitrazepam (specific activity = 84.5 Ci mmol^{-1}; New England Nuclear).

Receptor binding by autoradiography

Frozen coronal brain sections of 20 μm were cut on a cryostat, thaw-mounted onto gelatin-coated slides and stored at −70°C until the day of incubation. In-vitro autoradiography was performed on parallel sections for labelling 5-HT_{1A} (^{3}H-8-OH-DPAT), a_1-adrenergic (^{3}H-prazosin) and BZD (^{3}H-flunitrazepam) receptors in the presence or absence of indorenate. Experimental conditions for autoradiography experiments are summarized in Table 1.

Brain sections were pre-washed for 30 min at room temperature in the respective buffer to remove endogenous ligands. Then, they were incubated in a solution containing the specific radioligand in the presence or absence of 1 μM indorenate (a saturating concentration). Incubation was completed with two consecutive buffer washes (1 min each at 4°C). Finally, the slides were rinsed (3 s) in distilled water at 4°C and the sections were quickly dried under a gentle stream of cold air. The slides were arranged in X-ray cassettes together with tritium standards (Amersham), and apposed to radioactivity-sensitive film (Biomax-MR, Kodak) at room temperature (Table 1). Films were developed using developer D11 (Kodak) and fixer at room temperature. Optical densities of the autoradiograms were determined using a video-computer enhancement program (JAVA Jandel Video Analysis Software). The optical density of the standards was used to determine tissue radioactivity values for the accompanying tissue sections and to convert them to fmol (mg protein)$^{-1}$. Brain regions were identified according to the rat atlas of Paxinos & Watson (1998). For each structure, 10 optical density readings were taken from at least 2 sections, and they were averaged.

Binding was analysed in the cingulate, frontal, sensorimotor, piriform, temporal and entorhinal cortices, caudate putamen, amygdala nuclei (anterior, basolateral, central and medial nuclei), septal nucleus (only for ^{3}H-8-OH-DPAT binding) dentate gyrus, fields CA1–CA3 of dorsal and ventral hippocampus, thalamus, hypothalamus, substantia nigra pars compacta and reticulata, periaqueductal gray, dorsal and median raphe nucleui and locus coeruleus (only for ^{3}H-prazosin binding). These specific structures were chosen to evaluate receptor binding because of their possible role in numerous physiological responses.

Statistical analysis

Data were analysed by applying one-tailed paired Student's *t*-test. GraphPad Prism software version 4.00 for Windows was used, and $P < 0.05$ denoted significance.

Table 1 Experimental conditions for in-vitro receptor autoradiography experiments

Receptor	Ligand (nM) S.A.	Buffer pH 7.4–7.6	Incubation time/temp.	Exposure time/temp.	References
5-HT_{1A}	^{3}H-8-OH-DPAT 2 nM 135 Ci mmol^{-1}	170 mM Tris HCl +4 mM $CaCl_2$ +0.01% ascorbic acid	60 min/25°C	8 weeks/25°C	Pazos & Palacios (1985)
BZD	^{3}H-flunitrazepam 2 nM 84.5 Ci mmol^{-1}	170 mM Tris HCl	45 min/4°C	3 weeks/25°C	Rocha et al (1994)
a_1-Adrenergic	^{3}H-prazosin 2 nM 77.2 Ci mmol^{-1}	50 mM Krebs phosphate	60 min/25°C	12 weeks/25°C	Giroux et al (1999), Stephenson & Summers (1986)

S.A., specific activity.

Results

Effect of indorenate on 5-HT_{1A} receptor binding

The autoradiography experiments revealed that indorenate at 1 μM totally blocked the binding of 2 nM ^{3}H-8-OH-DPAT, because no signal was detected in the autoradiograms in any of the brain areas evaluated (Table 2).

Effect of indorenate on a_1-adrenergic receptor binding

Indorenate reduced ^{3}H-prazosin binding in the frontal (30%) and sensorimotor (32%) cortices as well as in the thalamus (21%) (Table 3). Non significant reduction of ^{3}H-prazosin binding was found in the dentate gyrus from dorsal hippocampus (19%), CA1 (15%) and CA2 (27%) from ventral hippocampus, basolateral amygdala (36%), caudate putamen (36%) and substantia nigra pars compacta (21%).

Table 2 ^{3}H-8-OH-DPAT binding (fmol (mg protein)$^{-1}$) in the presence or absence of indorenate (1 μM) in the rat brain

Brain structure	^{3}H-8-OH-DPAT	^{3}H-8-OH-DPAT + indorenate
Cortices		
Cingulate	64.5 ± 3.5	ND
Frontal	61 ± 2.2	ND
Sensorimotor	41.4 ± 3.3	ND
Piriform	145.5 ± 46.9	ND
Temporal	30.5 ± 4.4	ND
Entorhinal	69.3 ± 2.9	ND
Dorsal hippocampus		
Dentate gyrus	1308.8 ± 245.9	ND
CA1 field	574.3 ± 69.1	ND
CA2 field	56 ± 4	ND
CA3 field	856.6 ± 162.2	ND
Septal nucleus	2172.5 ± 74.5	ND
Ventral hippocampus		
Dentate gyrus	1609.4 ± 214.7	ND
CA1 field	494.2 ± 15.2	ND
CA2 field	483.2 ± 53.2	ND
CA3 field	454 ± 58.7	ND
Amygdala nuclei		
Anterior	40.5 ± 2.9	ND
Central	25.7 ± 4.2	ND
Medial	63.4 ± 4.5	ND
Basolateral	30.3 ± 4.1	ND
Caudate putamen	20.9 ± 1.6	ND
Thalamus	20.2 ± 3.8	ND
Hypothalamus	38.1 ± 3.3	ND
Periaqueductal gray	20.9 ± 4.5	ND
Raphe nuclei		
Dorsal	785.9 ± 151.2	ND
Median	95.3 ± 40.5	ND

The values are indicated as the mean ± s.e.m., n = 8. ND, non-detectable.

Table 3 ^{3}H-prazosin binding (fmol (mg protein)$^{-1}$) in the presence or absence of indorenate (1 μM) in the rat brain

Brain structure	^{3}H-Prazosin	^{3}H-Prazosin + indorenate	*P* value
Cortices			
Cingulate	555.9 ± 77.8	597.6 ± 81	0.2300
Frontal	457.2 ± 70.8	319.2 ± 41.9	0.0430
Sensorimotor	374.3 ± 32.4	253.6 ± 49.5	0.0238
Piriform	121.6 ± 27.5	169.1 ± 37.3	0.1013
Temporal	387.3 ± 39.3	422.1 ± 11.3	0.2239
Entorhinal	196.6 ± 48.8	229 ± 41.3	0.2952
Dorsal hippocampus			
Dentate gyrus	215.4 ± 44.9	175.4 ± 41	0.2190
CA1 field	173.2 ± 38.9	159.6 ± 36.4	0.3660
CA2 field	119.8 ± 25.1	134.1 ± 31	0.3701
CA3 field	144.4 ± 32.9	138.6 ± 33.2	0.4402
Ventral hippocampus			
CA1 field	326.5 ± 45	277.6 ± 44.2	0.2566
CA2 field	239.9 ± 46.4	174.9 ± 38.4	0.1372
CA3 field	244.1 ± 45.9	202.9 ± 41.1	0.2193
Amygdala nuclei			
Anterior	178.8 ± 41.5	172.3 ± 38.5	0.4346
Central	92.3 ± 2.5	90.9 ± 1.9	0.3241
Medial	155.8 ± 39.8	172.3 ± 37.6	0.3685
Basolateral	230.8 ± 44.9	206.5 ± 42.8	0.3657
Caudate putamen	145.2 ± 33.6	92.8 ± 2.6	0.0677
Thalamus	1174 ± 100.7	924.4 ± 122.4	0.0358
Hypothalamus	178.8 ± 41.8	176.2 ± 40.1	0.4742
Substantia nigra			
Pars reticulata	186.4 ± 45.5	172.5 ± 40.2	0.3727
Pars compacta	112.2 ± 23.9	88.2 ± 2.5	0.1544
Periaqueductal gray	281.1 ± 45.6	278.7 ± 45.5	0.4786
Raphe nuclei			
Dorsal	2079 ± 440.1	2841 ± 567.8	0.1784
Median	208.9 ± 45	193.3 ± 48.6	0.4148
Locus coeruleus	569.3 ± 89.7	542.4 ± 96.2	0.3630

The values are indicated as the mean ± s.e.m., n = 8. The data were analysed by Student's *t*-test.

Effect of indorenate on BZD receptor binding

Indorenate significantly decreased ^{3}H-flunitrazepam binding in the cingulate (16%) and piriform (18%) cortices as well as in the dorsal portion of the raphe nucleus (18%) (Table 4). Non-significant decrease of ^{3}H-flunitrazepam binding was found in the temporal cortex (11%), CA2 field from dorsal hippocampus (12%), CA3 field from ventral hippocampus (15%), susbtantia nigra pars compacta (10%) and periaqueductal gray (19%).

Discussion

In this study we found that indorenate reduces ^{3}H-prazosin and ^{3}H-flunitrazepam binding in specific rat brain regions.

At the beginnings of the 1980s, indorenate was described as a serotonergic analogue with cardiovascular effects (Hong 1981; Safdy et al 1982). Later, it was found

Table 4 ^{3}H-flunitrazepam binding (fmol (mg protein)$^{-1}$) in the presence or absence of indorenate (1 μM) in the rat brain

Brain structure	^{3}H-Flunitrazepam	^{3}H-Flunitrazepam + indorenate	*P* value
Cortices			
Cingulate	955.4 ± 26.5	801.2 ± 34.1	0.0061
Frontal	816.3 ± 26.5	740.3 ± 34.4	0.1048
Sensorimotor	823 ± 31.1	817.9 ± 26.1	0.4636
Piriform	714.9 ± 49.2	588.3 ± 61.3	0.0374
Temporal	798.6 ± 27.4	708.5 ± 64.6	0.1042
Entorhinal	625.9 ± 59.3	579.7 ± 52.7	0.3010
Dorsal hippocampus			
Dentate gyrus	763.7 ± 84.9	834.1 ± 36.6	0.2276
CA1 field	668.1 ± 61.6	655.7 ± 41	0.4341
CA2 field	512.1 ± 36.3	452.9 ± 43.9	0.1890
CA3 field	649.3 ± 58.9	602.8 ± 54.5	0.2492
Ventral hippocampus			
Dentate gyrus	814 ± 31.7	771.8 ± 62.4	0.2655
CA1 field	548.3 ± 47.2	564.8 ± 52.6	0.4009
CA2 field	452.3 ± 43	449.6 ± 35.9	0.4824
CA3 field	601.4 ± 49.4	512.8 ± 51.6	0.1174
Amygdala nuclei			
Anterior	716.1 ± 48	675.6 ± 55	0.2858
Central	375.3 ± 14.6	357.5 ± 10	0.1625
Medial	692.5 ± 63.5	691.3 ± 55.9	0.4934
Basolateral	716.8 ± 54.7	760.4 ± 56.1	0.3127
Caudate putamen	328.9 ± 12.5	312.5 ± 8.7	0.2181
Thalamus	334.3 ± 14.8	357.1 ± 11	0.1127
Hypothalamus	468.1 ± 44.8	518.4 ± 57.8	0.2663
Substantia nigra			
Pars reticulata	719.2 ± 69.5	695.5 ± 61.4	0.3850
Pars compacta	433.4 ± 55.1	390.4 ± 17.4	0.1696
Periaqueductal gray	644.9 ± 68	522.9 ± 61.7	0.0636
Raphe nuclei			
Dorsal	541.8 ± 43	445.2 ± 30	0.0282
Median	307 ± 10.3	281.6 ± 13.3	0.1148

The values are indicated as the mean ± s.e.m., n = 8. The data were analysed by Student's *t*-test.

to be a high-affinity 5-HT$_{1A}$ agonist with a K_d value of 15 nM (Hong 1981; Hoyer et al 1985). At present, it is known that indorenate induces antihypertensive, anxiolytic, antidepressant and anticonvulsant effects in experimental models through activation of 5-HT$_{1A}$ receptors (Hoyer et al 1985; Fernández-Guasti & López-Rubalcava 1990, 1998; Martínez-Mota et al 2002; López-Meraz et al 2005). Our autoradiography experiments revealed that indorenate displaces entirely ^{3}H-8-OH-DPAT from all brain areas evaluated. This finding corroborates its interaction with 5-HT$_{1A}$ receptors.

Interaction between brain noradrenergic and serotonergic systems is known (Bortolozzi & Artigas 2003; Mongeau et al 1997). Furthermore, it has been shown that the 5-HT$_{1A}$ receptor agonists 8-OH-DPAT and buspirone can modify cardiovascular parameters in-vitro or in-vivo through activation of vascular a_1-adrenergic receptors (Oakley & Jones 1983; Castillo et al 1993). Additionally, several studies indicate that other 5-HT$_{1A}$ receptor ligands, such as BMY7378, NAN-190 and WAY100635, show high affinity for a_1-adrenoceptors (Testa et al 1999; Yoshio et al 2001). Interestingly, the results from this study indicate that indorenate just decreases ^{3}H-prazosin binding in the frontal and sensorimotor cortices as well as the thalamus. Indorenate's effects in specific regions could be related to the existence of different subtypes of a_1-adrenoceptors with specific topographical distribution (Alonso-Llamazares et al 1995). Future experiments using selective radioligands for a_{1A}-,a_{1B}- and a_{1D}-adrenoceptors will test this hypothesis.

Studies support the functional interaction between GABA/BZD and 5-HT$_{1A}$ receptors (Guptarak et al 2004). It is known that buspirone increases ^{3}H-flunitrazepam binding in the cortex, cerebellum and hippocampus determined by in-vivo autoradiography (Oakley & Jones 1983). In-vivo administration of an anxiolytic dose of 8-OH-DPAT doubles the K_d value for the in-vivo binding of ^{3}H-flunitrazepam to rat cortical membranes and enhances the GABA-stimulated ^{36}Cl influx in rat corticohippocampal synaptoneurosomes (Sölderpalm et al 1997). The anxiolytic effects produced by some 5-HT$_{1A}$ receptor agonists, including indorenate, are blocked by flumazenil, a BZD receptor antagonist (Fernández-Guasti & López-Rubalcava 1998; López-Rubalcava et al 1992). In this study, indorenate decreased ^{3}H-flunitrazepam binding in the cingulate and piriform cortices, as well as in the dorsal raphe nucleus, suggesting that this drug may act on BZD receptors. The explanation for this region-dependent effect is unclear. It is well known that GABA$_A$ receptors are composed of five subunits deriving from 13 different genes (alpha1–6, beta1–beta3, gamma1–gamma3 and delta) (Pirker et al 2000). Depending on their subunit composition, these receptors exhibit distinct pharmacological and electrophysiological properties (Sieghart & Sperk 2002). Benzodiazepine pharmacology of the oligomeric GABA$_A$ receptor is dependent mainly on the nature of the alpha or gamma subunits (von Blankenfeld et al 1990; Bureau & Olsen 1993; Saxena & MacDonald 1996). Additionally, GABA$_A$ receptor subunits are heterogeneously distributed in the brain (Piker et al 2000). It is possible that decreases in ^{3}H-flunitrazepam binding induced by indorenate in particular brain regions may result from its interaction with specific subtypes of GABA$_A$/BZD receptors. Additional studies examining this possibility will add to the understanding of the relationship between indorenate and BZD receptors and its action in specific brain areas.

The decreased ^{3}H-prazosin and ^{3}H-flunitrazepam binding in the presence of indorenate may also be associated with a direct interaction of this drug with the a_1-adrenergic and BDZ sites (i.e., competition with the radioactive ligand for the receptors or allosterical changes in the affinity of the ligands for their receptors).

The fact that indorenate reduces ^{3}H-prazosin and ^{3}H-flunitrazepam binding in the cingulate, frontal, sensorimotor and piriform cortices, thalamus and the raphe nucleus, structures implicated in anxiety (Kalin et al 2005; de Medeiros et al 2005), depression (Lira et al 2003; Milak et al 2005), and epilepsy (Gutnick & Prince 1972; Chervin et al 1988; Fernández-Guardiola et al 1981), could have functional implications in its pharmacological effects, particularly those that do not depend on 5-HT$_{1A}$ receptors activation.

In conclusion, the findings from this study suggest that indorenate may interact with a_1-adrenergic and BZD receptors on selective brain areas. This situation may be associated with the pharmacological effects of indorenate in neurological disorders, such as anxiety, depression and epilepsy.

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