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Inhibition of brain [^{3}H]cimetidine binding by improgan-like antinociceptive drugs

Rebecca Stadel [a], Amanda B. Carpenter [a], Julia W. Nalwalk [a], Iwan J.P. de Esch [b], Elwin Janssen [b], Lindsay B. Hough [a,*]

[a] Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, NY, USA
[b] Leiden/Amsterdam Center for Drug Research, Vrije University Amsterdam, Amsterdam, The Netherlands

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ABSTRACT

[^{3}H]cimetidine, a radiolabeled histamine H_2 receptor antagonist, binds with high affinity to an unknown hemoprotein in the brain which is not the histamine H_2 receptor. Improgan, a close chemical congener of cimetidine, is a highly effective pain-relieving drug following CNS administration, yet its mechanism of action remains unknown. To test the hypothesis that the [^{3}H]cimetidine-binding site is the improgan antinociceptive target, improgan, cimetidine, and 8 other chemical congeners were studied as potential inhibitors of [^{3}H]cimetidine binding in membrane fractions from the rat brain. All compounds produced a concentration-dependent inhibition of [^{3}H]cimetidine binding over a 500-fold range of potencies (K_i values were 14.5 to >8000 nM). However, antinociceptive potencies in rats did not significantly correlate with [^{3}H] cimetidine-binding affinities ($r=0.018$, $p=0.97$, $n=10$). These results suggest that the [^{3}H]cimetidine-binding site is not the analgesic target for improgan-like drugs.

1. Introduction

The histamine H_2 receptor antagonist cimetidine exhibits high affinity, specific binding to a brain protein which is not the histamine H_2 receptor, but the identity and significance of this binding site have not been determined (Warrander et al., 1983; Smith et al., 1980; Burkard, 1978). The nature of the [^{3}H]cimetidine-binding site is of considerable interest due to the non-histamine H_2 receptor-mediated actions of cimetidine, which include antinociception (Netti et al., 1984; Hough et al., 1997) and neurotoxicity (Shimokawa et al., 1996; Amabeoku and Chikuni, 1993; Edmonds et al., 1979). Recently, the [^{3}H]cimetidine-binding site was pharmacologically characterized in detail as a heme-containing protein, possibly a member of the cytochrome P450 superfamily (Stadel et al., 2008).

Improgan (Table 1) is a chemical congener of cimetidine that shares cimetidine's antinociceptive properties following intracerebroventricular administration, but lacks affinity for the histamine H_2 receptor (Li et al., 1996). Improgan produces antinociception in several pain models, suggesting a favorable pre-clinical profile (Li et al., 1997; Bannoura et al., 1998). Evaluation of over 110 possible targets, including various ion channels and G protein-coupled receptors (Hough et al., 2000a and unpublished data), has not identified the site of improgan antinociceptive action. Improgan lacks affinity for many known antinociceptive receptors, including all known histamine (Mobarakeh et al., 2003), opioid (Hough et al., 2000b), and cannabinoid receptors (Hough et al., 2002). Improgan acts in the brain stem to stimulate descending pain-relieving mechanisms which may include supraspinal cannabinoid receptors and spinal α_2 adrenergic receptors, but the drug lacks affinity for these receptors as well (Hough et al., 2002;Hough et al., 2000a). Failure to identify the antinociceptive target for improgan has prevented further clinical development.

We recently found that improgan competes with [^{3}H]cimetidine binding in the rat brain (Hough et al., 2007). The same study reported the discovery of CC12 (i.e. 4(5)-((4-iodobenzyl)thiomethyl)-1H-imidazole), a new cimetidine congener with nanomolar affinity ($K_i=9.5$ nM) for the [^{3}H]cimetidine-binding site. CC12 was also found to inhibit improgan antinociception, suggesting the possibility that the [^{3}H]cimetidine-binding site is the molecular target for improgan-like antinociceptive drugs. In order to characterize further the pharmacological properties and potential antinociceptive relevance of the [^{3}H]cimetidine-binding site, the effects of improgan, cimetidine and 8 additional antinociceptive congeners of improgan (Table 1) have been studied presently as inhibitors of [^{3}H]cimetidine binding.

2. Methods

2.1. Chemicals

Unless noted otherwise, the compounds in Table 1 were synthesized as described recently (Hough et al., 2006). Improgan was synthesized as described (Mobarakeh et al., 2003). Cimetidine and

* Corresponding author. Center for Neuropharmacology and Neuroscience, Albany Medical College MC-136, 47 New Scotland Ave, Albany, NY 12208, USA. Tel.: +1 518 262 5786; fax: +1 518 262 5799.
E-mail address: houghl@mail.amc.edu (L.B. Hough).

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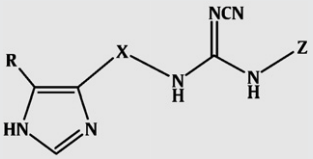
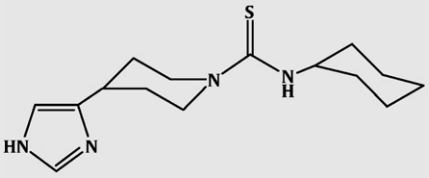
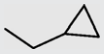
Table 1
Chemical structures, [^{3}H]cimetidine-binding affinities, and antinociceptive potencies of cimetidine and improgan congeners. Except for thioperamide (structure of which is given at the right), chemical structures for all compounds in the table refer to the generic, improgan-like structure given at the left.



Improgan-Like



Thioperamide

#	Drug	R	X	Z	3HClM IC_{50} (nM) [a]	3HClM K_i (nM) [b]	Antinociceptive ED_{50} (nmol) [c]
1	VUF6914	H	$-(CH_2)_8-$	$-CH_3$	25.4	14.5	81.9
2	VUF6913	H	$-(CH_2)_5-$	$-CH_3$	48.6	27.8	105.5
3	Cimetidine	CH_3	$-CH_2-S-(CH_2)_2-$	$-CH_3$	53.6	30.6	417.3 [d]
4	VUF5733	H	$-(CH_2)_2-$	$-CH_3$	156.0	89.2	137.3
5	Thioperamide	–	–	–	175.3	100.2	> 1700 [e]
6	VUF5651	CH_3	$-(CH_2)_4-$	$-CH_3$	214.1	122.4	105.1
7	VUF5420	H	$-(CH_2)_4-$	$-CH_3$	269.8	154.2	81.7
8	VUF6990	H	$-(CH_2)_3-$		742.7	424.5	86.6 [e]
9	Improgan	H	$-(CH_2)_3-$	$-CH_3$	1367.4	781.6	220.6
10	CC10	H		$-CH_3$	>15,000	>8000	106.1

[a] [^{3}H]cimetidine (3HClM)-binding IC_{50} values from Fig. 1.
[b] K_i values calculated from respective IC_{50} values (K_D for [^{3}H]cimetidine = 67 nM, [Stadel et al., 2008]).
[c] Unless noted otherwise, ED_{50} values are from hot plate nociceptive data measured 5 min following intracerebroventricular administration in rats housed under a normal light–dark cycle (Hough et al., 2006).
[d] Value from data taken 10 min after intracerebroventricular administration of cimetidine in rats housed under reverse (dark-cycled) conditions (Li et al., 1996).
[e] Values from Fig. 2.

thioperamide maleate were purchased from Tocris Bioscience (Ellisville, MO). Burimamide was kindly provided by Dr. Mark Wentland (Rennselaer Polytechnic Institute, Troy, NY). CC10 was kindly provided by Dr. James Phillips (Curragh Chemistries, Cleveland, OH). Compounds in salt form were dissolved in saline. Free base forms were dissolved in dilute HCl, titrated to pH 5.5–6.0 and diluted with saline.

2.2. Synthesis of VUF6990

1-Cyano-3-[3-(1*H*-imidazol-4-yl)-propyl]-2-methyl-isothiourea (0.90 mmol, 200 mg, prepared according to Hough et al., 2006) was dissolved in cyclopropylmethylamine (3 ml) and heated under microwave conditions (30 min, 100 °C, 150 W). Co-evaporation with chloroform yielded the crude product which was purified over silica (acetone: methanol 9:1). The resulting colorless oil was crystallized with acetone/hexane, which gave the product (base) as a white foam (isolated yield 23%, purity 98% by NMR). $\delta_{(H)}$ (CD_3OD, 200 MHz) 7.57 (s, 1H), 6.82 (s, 1H), 3.24 (t, J = 9.0 Hz, 2H), 3.06 (d, J = 7.6 Hz, 2H), 2.62 (t, 7.1 Hz, 2H), 1.87 (m, 2H), 1.07 (m, 1H), 0.67–0.13 (m, 4H); $\delta_{(C)}$ (CD_3OD, 200 MHz) 3.9, 11.6, 24.9, 30.3, 42.3, 47.4, 117.3, 120.4, 135.9, 138.1, and 161.2; *m/z* (ESI) calculated for $C_{12}H_{18}N_6$:246.31, found: 247.1.

2.3. Animals

Male Sprague–Dawley rats (250–330 g, Taconic Farms, Germantown, NY) were used for all studies. They were housed in groups of 3–4 on a 12-h light/dark cycle (lights on from 0700 to 1900) with food and water ad libitum. All animal experiments were approved by the Institutional Animal Care and Use Committee of Albany Medical College.

2.4. Isolation of brain membrane fractions

Homogenates were prepared as recently described (Stadel et al., 2008). Rats were euthanized with an overdose of CO_2 or pentobarbital and brains were rapidly removed. In some cases frozen brains were purchased (Taconic Farms, Germantown, NY). Brains were homogenized (polytron) in 10 volumes of homogenate buffer (100 mM Tris–HCl, 0.5 mM EDTA, pH 7.4), and centrifuged (26,000 × *g* for 15 min). Pellets were resuspended in buffer with a glass–teflon homogenizer, recentrifuged, and the resulting pellets stored at −80 °C. On the day of assay, pellets were washed in assay buffer (100 mM Tris–HCl, pH 7.4), centrifuged (26,000 × *g* for 10 min), resuspended in a volume 5 times the wet weight of the original tissue, and analyzed for [^{3}H] cimetidine-binding activity.

2.5. Radioligand binding

[^{3}H]cimetidine-binding experiments were performed following Smith et al. (1980) as recently described (Stadel et al., 2008). Resuspended crude membrane pellets (360–470 μg of rat brain protein) were incubated in a total volume of 0.1 ml containing 50 nM [^{3}H] cimetidine (20–25 Ci/mmol, G.E. Healthcare, Piscataway, NJ), various concentrations of competing ligand, and assay buffer for 60 min on ice. To evaluate non-specific binding, burimamide (30 μM) or cimetidine (10 μM) was added. Following incubation, samples were filtered through GF/B filters. Filters were rinsed three times with 1.5 ml of ice-cold assay buffer, placed in 5 ml of Ecoscint scintillation fluid, and counted in a scintillation counter. Protein content was determined using the bicinchoninic acid method (Pierce Chemical, Rockford, IL). For competition studies, percent specific binding was calculated using the following formula: [(drug − non-specific)/(total − non-specific) × 100], where drug and total indicate the amount of binding in the presence and absence of competing ligand, respectively.

2.6. Intracerebral surgery

Surgeries, drug treatments, and antinociceptive testing of improgan and analogs were performed as recently described (Hough et al., 2006). Briefly, rats were anesthetized with pentobarbital (25 mg/kg, i.p.), supplemented with isofluorane. Guide cannulas were stereotaxically implanted aimed toward the left lateral ventricle (−0.8 AP, 1.5 ML,