2′-Substituted analogs of cocaine: synthesis and dopamine transporter binding potencies

A series of 2′-substituted cocaine analogs (4–8) was prepared and evaluated in an in vitro dopamine transporter (DAT) binding assay. Compounds 4–7 were prepared by esterifying the 3β-hydroxy group of ecgonine methyl ester (3) using the appropriate acid chloride in the presence of Et3N and benzene. Compound 3 was obtained from cocaine (1) by hydrolysis using 1N HCl to afford ecgonine-HCl which was subjected to acid catalyzed esterification using methanol saturated with HCl gas. Compound 8 was obtained by hydrogenation of 7 using H2/Pd-C. The IC50 values were calculated from displacement experiment of the radioligand [3H]WIN-35,428 (2). 2′-Aminococaine (8) showed high binding affinity to the DAT (14- and 1.3-fold more active than cocaine and the radioligand 2, respectively). These results, along with previous results, emphasize the importance of a hydrogen-bond donor group at the 2′-position of cocaine to enhance binding affinity to the DAT.

Key Words: Cocaine analogs; Substituted cocaines; Cocaine antagonists; Dopaminergic system

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Compounds 4–8 were tested for their abilities to displace \(^{[3}\text{H}]\)WIN-35,428 and the IC\(_{50}\) values for inhibiting 4 nM of the radioligand binding to DAT are listed in Table 2.

The prepared compounds had 2′-substituents of different chemical nature as follows: (a) a bulky group, 4, (b) an electron-donating group, 5 and 6, (c) an electron-withdrawing group, 7, and (d) a hydrogen-bond donor, 8.

Compounds 6 (2′-SH) and 8 (2′-NH\(_2\)) were prepared in view of the high binding potency shown by 2′-hydroxycocaine which has an IC\(_{50}\) (mean ± SEM) value of 25 ± 4 nM (about 10-fold more active than cocaine which has an IC\(_{50}\) value of 249 ± 37 nM) and is nearly of equal potency to the radioligand WIN-35,428 (2)\(^{[21,23]}\). As shown in Table 2, 2′-NH\(_2\) cocaine had a binding potency with an IC\(_{50}\) value of 18 ± 2 nM, which is about 14- and 1.3-fold more active than cocaine and 2, respectively. It was postulated that the hydroxyl group, and similarly the amino group, may engage in an intermolecular hydrogen-bonding with the serine residues at the acceptor site of dopamine transporter \(^{[21]}\). The high binding potency exhibited by 2′-OH and 2′-NH\(_2\) (unusual for the benzyl ester class of tropanes) emphasized the importance of a hydrogen-bond donor group at this position in enhancing binding to DAT. These results are in accord with previous work published by our group \(^{[23]}\). This work included a bulky group (2′-CH\(_3\)), an electron-donating group (2′-OCOCH\(_3\)), a hydrogen-bond acceptor group (2′-F) and a hydrogen-bond donor group (2′-OH), which had IC\(_{50}\) values (mean ± SEM) of 251 ± 96, 70 ± 1, 604 ± 67 and 25 ± 4 nM, respectively.

Acknowledgment

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Experimental

General

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Midwest MicroLab LTD, Indianapolis, IN, USA. NMR spectra were recorded on a Varian XL-300 spectrometer. All organic reagents were obtained from Aldrich Co. and were used without further purification. Silica gel (200–400 mesh, 60 Å) used for column chromatography was obtained from Aldrich and silica gel chromatographic sheets with a fluorescent indicator used for thin layer chromatography (TLC) were obtained from Eastman Kodak Co., Rochester, NY. \(^{[3}\text{H}]\)WIN-35,428 was obtained from Dupont-New England Nuclear, Boston, MA. Homogenization of the striata was performed using a Polytron Homogenizer. Kinematic Kriens-Luzern, Switzerland. Centrifugation of the membrane homogenate was carried out using a DuPont Sorvall RC-5 superspeed refrigerated centrifuge (rotor SS-34). Filtration of the bound-membrane was carried out using a Brandel Cell Harvester. Counting of the activity of the receptor-bound ligand was performed using a Beckman LS 1701 liquid scintillation counter.

Table 2. Dopamine transporter binding affinities of 4–8.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>IC(_{50}) (nM)</th>
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<tbody>
<tr>
<td>Cocaine (1)</td>
<td>249 ± 37</td>
</tr>
<tr>
<td>[^{[3}\text{H}])WIN-35,428 (2)</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>4</td>
<td>8162 ± 152</td>
</tr>
<tr>
<td>5</td>
<td>46654 ± 840</td>
</tr>
<tr>
<td>6</td>
<td>3224 ± 172</td>
</tr>
<tr>
<td>7</td>
<td>8440 ± 986</td>
</tr>
<tr>
<td>8</td>
<td>18 ± 2</td>
</tr>
</tbody>
</table>

IC\(_{50}\) values are mean ± standard error of the mean (SEM) of two to three experiments performed in triplicate.
Chemistry

General procedures for the preparation of 2’-cocaine analogs

Ecgonine methyl ester-HCl (3) was obtained from cocaine by hydrolysis with 1N HCl and the produced ecgonine-HCl was subjected to acid catalyzed esterification by stirring ecgonine-HCl overnight with MeOH saturated with HCl gas. To the free base of 3 (0.30 g, 1.50 mmol) dissolved in dry C6H6 (15 mL) was added Et3N (1 mL, 10 mmol). To the stirred solution were added, under dry N2, 2-phenyl-, methoxy-, mercapto-, or nitro benzoyl chloride (2.25 mmol). The reaction mixtures were stirred, under dry N2, at 40 °C overnight. The reactions were stopped and the benzene layer was washed with H2O (10 mL) for 3 times and the free base of 3 was obtained from cocaine by hydrolysis with 1N HCl. The oils were purified on a silica gel column, cf. Table 1, to afford the pure oils. The oils were converted to the tartrate salt and recrystallized from methanol/ether. Elemental analyses (C, H and N) were within ±0.4 % of calculated values.

1H-NMR (D2O, δ)

4: 7.66–7.63 (m, 1H, C(6')H), 7.52–7.47 (m, 1H, C(4')H), 7.35–7.30 (m, 1H, C(5')H), 7.28 (s, 5H, C6H5), 7.15–7.12 (m, 1H, C(3')H), 5.17–5.13 (m, 1H, C(3)H), 3.97–3.95 (m, 1H, C(1)H), 3.75 (m, 1H, C(5)H), 3.49 (s, 3H, COOCH3), 3.25–3.24 (m, 1H, C(2)H), 2.64 (s, 3H, NCH3), 2.30–2.28 (m, 4H, C(4, 7)H), 2.05–2.00 (m, 2H, C(6)H).

5: 7.65–7.62 (dd, 1H, C(6')H), 7.52–7.46 (m, 1H, C(4')H), 7.35–7.30 (m, 1H, C(5')H), 7.06–7.04 (d, 1H, C(3')H), 6.95–6.90 (m, 1H, C(5')H), 5.41–5.33 (m, 1H, C(3)H), 4.08–4.06 (m, 1H, C(1)H), 3.94–3.92 (m, 1H, C(5)H), 3.75 (s, 3H, COOCH3), 3.50 (s, 3H, Ar-OCH3), 3.25–3.24 (m, 1H, C(2)H), 2.72 (s, 3H, NCH3), 2.33–2.22 (m, 4H, C(4, 7)H), 1.93–1.89 (m, 2H, C(6)H).

6: 7.76 (m, 1H, C(6')H), 7.50 (m, 2H, C(4', 5')H), 7.34–7.31 (m, 1H, C(3')H), 5.41–5.33 (m, 1H, C(3)H), 4.05–4.03 (m, 1H, C(1)H), 3.91–3.85 (m, 1H, C(5)H), 3.51 (s, 3H, COOCH3), 3.47–3.45 (m, 1H, C(2)H), 2.66 (s, 3H, NCH3), 2.29–2.05 (m, 4H, C(4, 7)H), 2.04–1.98 (m, 2H, C(6)H).

7: 7.88–7.85 (m, 1H, C(3')H), 7.70–7.62 (m, 3H, C(4', 5', 6')H), 5.47–5.43 (m, 1H, C(3)H), 4.05–4.03 (m, 1H, C(1)H), 3.90 (m, 1H, C(5)H), 3.46 (s, 3H, COOCH3), 3.33–3.00 (m, 1H, C(2)H), 2.67 (s, 3H, NCH3), 2.30–2.28 (m, 4H, C(4, 7)H), 2.05–2.00 (m, 2H, C(6)H).

3β-[(Z′-Aminobenzoyl)oxy]-1R-(exo,exo)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester

To a 100 mL sealed round-bottomed flask under vacuo were attached to the flask while it was under vacuum to allow hydrogen to be sucked into the flask. The mixture was stirred for 24 h. The crude product was filtered over celite and the solvent was evaporated to obtain (0.27 g, 97% yield) of a pure oil. The oil was converted to the tartrate salt and recrystallized from methanol/ether. Elemental analyses (C, H and N) were within ±0.4% of calculated values.

1H-NMR (D2O, δ)

8: 7.57–7.54 (d, 1H, C(6')H), 7.26–7.19 (t, 1H, C(4')H), 6.72–6.69 (d, 1H, C(3')H), 6.59–6.57 (t, 1H, C(5')H), 5.42–5.34 (m, 1H, C(3)H), 4.04–4.02 (m, 1H, C(1)H), 3.88 (m, 1H, C(5)H); 3.74 (s, 3H, COOCH3); 3.46–3.43 (m, 1H, C(2)H); 2.69 (s, 3H, NCH3); 2.24–2.20 (m, 4H, C(4, 7)H); 2.01–2.00 (m, 2H, C(6)H).

Dopamine transporter binding assays

The binding was performed in rat striatal tissue using the method of Reith.[24]

Materials: [3H]WIN-35,428 was obtained from Dupont-New England Nuclear, Boston, MA.

Preparation of test substances: stock solutions of the test substances were prepared freshly by dissolving them in the incubation buffer (phosphate buffer pH 7.4).

Phosphate buffer: obtained by mixing 35 mM NaH2PO4 and 17.5 mM Na2HPO4 to obtain pH 7.4.

Membrane preparation: Whole brains from male Sprague-Dawley rats weighing from 350–400 g (Sasco Inc, Wilmington, MA) were rapidly harvested after decapitation with a guillotine. The striata were isolated and homogenized using a Polytron Homogenizer (setting 6 for 15 s) in ice-cold 0.32 M sucrose solution (1.5 mL/100 mg of tissue). The homogenizer and blade were then rinsed with twice the volume of 0.32 M sucrose solution which was added to the homogenate. The combined mixture was centrifuged at 3,000 rpm for 10 min at 4 °C. The resulting supernatant was subsequently centrifuged at 13,000 rpm for 20 min at 4 °C to obtain a pellet (P2), which was homogenized in ice-cold 35 mM phosphate buffer.

Each assay tube contained 130 µL buffer or buffer plus 10 nM of unlabeled test compound (1×10–10 to 100×10–6 M). [3H]WIN-35,428 in the same buffer (20 µL, 4 nM) and 50 µL of membranes (4 mg/mL) to a total volume of 200 µL. (--)Cocaine (100 µM) was used for non-specific binding. Assays performed in triplicate were incubated for 2 h in an ice bath and terminated by rapid filtration through Whatman GF/B glass fiber filters presoaked for 30 min in 0.05% polylysine solution. Membranes were rapidly washed three times with ice-cold buffer. Filters containing membrane-bound radioligand were added to vials containing 10 mL of scintillation fluid (Ecolume, Costa Mesa, CA), stored overnight, and counted for 5 min on a Beckman LS 1701 liquid scintillation counter.

Calculation of IC50: IC50 values were determined from competition curves of twelve points using the curve-fitting program EBDA (Biosoft software, Ferguson, MO). Mean values and standard errors were calculated from two to three assays for each test compound.

References
