Some pharmacological properties of Wy 27127 a more selective alpha\textsubscript{2}:alpha\textsubscript{1}-adrenoceptor antagonist than idazoxan in vitro

S. J. Bill, A. Boniface, F. Haroun, R. P. McAdams, N. Lattimer, and K. F. Rhodes
Wyeth Research (UK) Ltd, Huntercombe Lane South, Taplow, Maidenhead, Berkshire, SL6 0PH, UK

Summary. Wy 27127 and idazoxan were approximately equipotent as antagonists at alpha\textsubscript{2}-adrenoceptors as estimated by their ability to block clonidine-induced inhibition of electrically-evoked contractions of the rat isolated vas deferens.

Idazoxan was seven times as potent as Wy 27127, as an antagonist at alpha\textsubscript{1}-adrenoceptors as indicated by blockade of methoxamine-induced contractions of the rat isolated anococcygeus muscle.

Thus, the alpha\textsubscript{2}:alpha\textsubscript{1} selectivity ratio, as calculated from these tests was 407 for Wy 27127 and 76 for idazoxan.

Wy 27127 and idazoxan were equipotent in enhancing stimulation-evoked overflow of tritium from rabbit isolated pulmonary arteries preloaded with [3H]-noradrenaline as expected for alpha\textsubscript{2}-adrenoceptor antagonists. At higher concentrations both compounds reduced the stimulation-evoked contraction of the pulmonary artery but idazoxan was 15 times as potent as Wy 27127 in this respect.

Neither compound had marked antagonist actions at 5-hydroxytryptamine (D), muscarinic, presynaptic dopamine or histamine (H\textsubscript{1}) receptors or at beta\textsubscript{1}-adrenoceptors.

Thus, idazoxan and Wy 27127 were equipotent alpha\textsubscript{2}-adrenoceptor antagonists in vitro, however, the alpha\textsubscript{2}:alpha\textsubscript{1} selectivity of Wy 27127 was considerably greater than that of idazoxan by virtue of weaker alpha\textsubscript{1}-adrenoceptor antagonism.

Key words: Alpha\textsubscript{1}/alpha\textsubscript{2}-adrenoceptor antagonism – Wy 27127 – Idazoxan

Methods

General. Unless otherwise stated all experiments were conducted using Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.8; NaHCO\textsubscript{3}, 25.0; MgSO\textsubscript{4}, 1.2; KH\textsubscript{2}PO\textsubscript{4}, 1.2; glucose, 11.1; CaCl\textsubscript{2}, 2.5, gassed with a mixture of 5% CO\textsubscript{2} in oxygen and maintained at 37°C. Rats, male, Sprague-Dawley (200–250 g) were killed with a blow to the head. Guinea pigs, male Hartley strain (250–350 g) were killed by cervical dislocation. Antagonists were added to the Krebs solution reservoir and equilibrated with the tissues for 30 min unless otherwise stated.

The details of subsequent methods with the exception of the use of the rabbit rectococcygeus muscle in the study of presynaptic dopamine receptors (which is described in full) were described by Lattimer et al. (1984) and are only briefly outlined here.

alpha\textsubscript{2}-Adrenoceptor antagonism on the rat isolated vas deferens. A section (2 cm) was cut from the prostatic region of the vas deferens and suspended in an organ bath (6 ml) at 34.5°C. Platinum ring electrodes were used for field stimulation.
Table 1. \(\alpha_1\)- and \(\alpha_2\)-adrenoceptor antagonism by Wy 27127 and idazoxan in the isolated vas deferens and anococcygeus muscle of the rat

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vas deferens ((\alpha_2))</th>
<th>B-HT 933</th>
<th>Anococcygeus ((\alpha_1))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clonidine</td>
<td>Methoxamine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(pA_2) Slope (r) (n)</td>
<td>(pA_2) Slope (r) (n)</td>
<td>(pA_2) Slope (r) (n)</td>
</tr>
<tr>
<td>Wy 27127</td>
<td>7.93*</td>
<td>7.63*</td>
<td>5.32*</td>
</tr>
<tr>
<td></td>
<td>(7.68 - 8.34)</td>
<td>(7.46 - 7.86)</td>
<td>(5.12 - 5.84)</td>
</tr>
<tr>
<td></td>
<td>(0.74 - 1.18)</td>
<td>(0.83 - 1.17)</td>
<td>(0.46 - 1.28)</td>
</tr>
<tr>
<td>Idazoxan</td>
<td>8.04*</td>
<td>8.20*</td>
<td>6.16*</td>
</tr>
<tr>
<td></td>
<td>(7.86 - 8.26)</td>
<td>(7.97 - 8.50)</td>
<td>(5.91 - 6.52)</td>
</tr>
<tr>
<td></td>
<td>(0.83 - 1.07)</td>
<td>(0.75 - 1.01)</td>
<td>(0.77 - 1.14)</td>
</tr>
</tbody>
</table>

\(pA_2\) values, correlation coefficients \((r)\) and slopes calculated by regression analysis of Schild plots are shown. 95\% confidence limits are shown in brackets. \(n\) is number of tissues used.

* Previously reported (Lattimer and Rhodes 1985)

Concentrations of antagonists tested: \(a\) \(10^{-7}\), \(3 \times 10^{-7}\), \(10^{-6}\) M; \(b\) \(10^{-5}\), \(3 \times 10^{-5}\) M; \(c\) \(3 \times 10^{-6}\), \(10^{-5}\), \(1.1 \times 10^{-4}\) M

Increasing concentrations (Fig. 2) of idazoxan or Wy 27127 were included in the Krebs solution 12 min before \(S_5\), \(S_7\), \(S_9\) and \(S_{11}\). Drug effects were assessed from stimulation periods \(S_6\), \(S_8\), \(S_{10}\) and \(S_{12}\) (Fig. 2). Thus, four increasing concentrations of an antagonist were tested in each tissue.

A group of tissues stimulated as above but not exposed to \(\alpha_2\)-adrenoceptor antagonists served as controls.

5-HT antagonism on the rat isolated ileum. Non-cumulative concentration-response curves to 5-HT were obtained using a 30 s contact time and 2.5 min wash periods between each addition of 5-HT. Isometric contractions were recorded. Idazoxan and Wy 27127 were tested at \(10^{-6}\), \(3 \times 10^{-6}\) and \(10^{-5}\) M. Three increasing concentrations of one antagonist were tested in each tissue.

Histamine and acetylcholine (\(ACh\)) antagonism on the guinea pig isolated ileum. Non-cumulative concentration-response curves to histamine and \(ACh\) were obtained in different tissues using isotonic recording (1 g load) with a 30 s contact time and 2.5 min wash periods between each addition of agonist. Idazoxan and Wy 27127 were tested at \(10^{-5}\) M.

\(\beta\)-Adrenoceptor antagonism on guinea pig isolated atria. Atria were suspended in Ringer-Locke solution containing (mM): NaCl, 154.0; KCl, 5.7; glucose, 5.5; NaHCO\(_3\), 5.9; CaCl\(_2\), 1.1 and ascorbic acid 0.1 at 32\°C gassed with oxygen. Spontaneous atrial rate was recorded. Cumulative concentration-response curves to the chronotropic effect of isoprenaline were obtained using a 3 min contact time at each concentration. Idazoxan and Wy 27127 were tested at \(10^{-6}\), \(3 \times 10^{-6}\) and \(10^{-5}\) M. Three increasing concentrations of one antagonist were tested in each tissue.

Antagonism of dopamine on the rabbit isolated, field stimulated rectococcygeus muscle. The method used was similar to that of Drew and Hilditch (1984). Rectococcygeus muscles were removed from male, New Zealand white rabbits (2.5 - 3.0 kg) under pentobarbitone anaesthesia (45 mg/kg i.v.). They were divided into two equal sections (2 cm in length) and suspended in organ baths (6 ml) at 32\°C under a 1 g of tension. Krebs solution containing desipramine \((5 \times 10^{-7}\) M\), indomethacin \((5 \times 10^{-6}\) M\), corticosterone \((4 \times 10^{-5}\) M\), propranolol \((10^{-6}\) M\) and ascorbic acid \((10^{-4}\) M\), gassed with a 5\% CO\(_2\)/95\% O\(_2\) mixture was used. Platinum ring electrodes were positioned one