Electrophysiological Comparison of 5-Hydroxytryptamine\textsubscript{1A} Receptor Antagonists on Dorsal Raphe Cell Firing\textsuperscript{1}

LYNN P. MARTIN, DAVID M. JACKSON, CARIN WALLSTEN and BARBARA L. WASZCZAK

Department of Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts (L.P.M., B.L.W.); and Department of Pharmacology, Astra Arcus AB, Preclinical R&D, Södertälje, Sweden (D.M.J., C.W.)

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ABSTRACT

Single-unit recording studies were undertaken in chloral hydrate-anesthetized rats to compare the effects on dorsal raphe cell firing of several putative 5-hydroxytryptamine (HT)\textsubscript{1A} receptor antagonists, including WAY 100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexane-carboxamide), p-MPPI (4-(2-methoxyphenyl)-2-[2-[(N-(2'-pyridinyl)-p-iodobenzamido)ethyl]piperazine], and two newly described 5-HT\textsubscript{1A} receptor antagonists, NDL-249 ([R]-3-[N-propylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide) and NAD-299 ([R]-3-N,N-dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide). Consistent with a 5-HT\textsubscript{1A} receptor antagonist profile, pretreatment with an approximately equimolar (0.02–0.03 μmol/kg) i.v. dose of each compound caused a significant rightward shift in the dose-response curve for 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetrain]. Antagonist potency was clearly highest for NAD-299 and WAY 100635, which caused shifts roughly 3 times greater than those for either p-MPPI or NDL-249 (ED\textsubscript{50} for 8-OH-DPAT, 1.3 ± 0.3 μg/kg; after NAD-299, 18.2 ± 1.0 μg/kg; after WAY 100635, 16.9 ± 2.9 μg/kg; after NDL-249, 6.0 ± 1.2 μg/kg; after p-MPPI, 4.7 ± 1.1 μg/kg). In separate studies, each of the antagonists was administered alone in increasing cumulative doses to evaluate whether they possessed intrinsic agonist activity in this system. At doses below 0.01 μmol/kg, none of the drugs altered firing by more than ±20% basal rates. At higher doses (>0.1 μmol/kg), WAY 100635, NAD-299, and NAD-299 caused a dose-dependent suppression of dorsal raphe cell firing (ED\textsubscript{50} = 0.6 ± 0.2, 0.7 ± 0.3, and 0.9 ± 0.4 μmol/kg, respectively). However, the ED\textsubscript{50} values for inhibition by these drugs were roughly 30 times higher than the doses that antagonized effects of 8-OH-DPAT. Moreover, the inhibition by all three antagonists (but not 8-OH-DPAT) was readily reversed by α-amphetamine (3.2 mg/kg i.v.), a releaser of norepinephrine, suggesting that these effects were likely due to alpha adrenergic receptor blockade rather than to 5-HT\textsubscript{1A} receptor agonism. Thus, it was concluded that WAY 100635, NAD-299, NDL-249, and p-MPPI all fulfill criteria as 5-HT\textsubscript{1A} receptor antagonists lacking intrinsic efficacy in the dorsal raphe system. The newly described compound NAD-299 exhibits antagonist potency comparable to that of WAY 100635 in this electrophysiological assay.

The 5-hydroxytryptamine (HT)\textsubscript{1A} receptor has been implicated as the site mediating the anxiolytic effect of azapirone drugs such as buspirone, gepirone, ipsapirone, and tandospiron (Traber and Glaser, 1987; Chojnacka-Wojcik and Przegalinski, 1991, Martin et al., 1991). More recently, this receptor has been suggested as a target for pharmacological intervention not only for the treatment of anxiety but also for disorders such as depression, schizophrenia, and dementia (Fletcher et al., 1993). The potentially widespread involvement of serotonin receptors in neuropsychiatric disorders underlies the current surge in interest in deciphering the physiological roles of 5-HT\textsubscript{1A} receptors. Although this effort was greatly facilitated by identification of the first 5-HT\textsubscript{1A} receptor-selective agonist, 8-hydroxy-2-(di-n-propylamino)tetrain (8-OH-DPAT; Gozlan et al., 1983), a full characterization of 5-HT\textsubscript{1A} receptor pharmacology has been impeded by the lack of selective antagonists.

A number of laboratories have developed putative 5-HT\textsubscript{1A} receptor antagonists. The first generation of these compounds later were proved to be nonselective, behaved as “partial” 5-HT\textsubscript{1A} receptor agonists, or both (Hillver et al., 1990; Bjork et al., 1991; Claustre et al., 1991). Confusion has also arisen due to the use of different pharmacological models in examining the functional activity of 5-HT\textsubscript{1A} receptor ligands. Because 5-HT\textsubscript{1A} receptors serve as both somatodendritic autoreceptors on serotonergic neurons (Sotelo et al., 1990), as well as postsynaptic receptors in several brain regions, drug effects can vary widely depending on whether the assay assesses a presynaptic versus a postsynaptic function. For instance, a number of compounds display only

antagonist activity in models of postsynaptic 5-HT$_{1A}$ receptor function but exhibit weak to moderate “partial” agonist properties in models of somatodendritic 5-HT$_{1A}$ autoreceptor function (Meller et al., 1990; Claustre et al., 1991; Cox et al., 1993, Fornal et al., 1994a, b, 1996).

Recently, a group of compounds were developed that are claimed to behave as pure 5-HT$_{1A}$ receptor antagonists (i.e., compounds that are devoid of intrinsic activity in tests of either presynaptic or postsynaptic 5-HT$_{1A}$ Receptor activity). We have evaluated several of these drugs for their effects on the extracelluar, single-unit activities of rat dorsal raphe neurons, a standard in vivo electrophysiological assay for evaluating compounds with 5-HT$_{1A}$ receptor agonist or antagonist activity. The firing of dorsal raphe serotonergic neurons has been shown to be suppressed by systemic administration or iontophoretic or bath application of 5-HT$_{1A}$ receptor antagonists (Rogawski and Aghajanian, 1981; Sprouse and Aghajanian, 1987; Aghajanian et al., 1990), and this response is blocked by 5-HT$_{1A}$ antagonists (Forster et al., 1995, 1996). The inhibition of firing by serotonin agonists was found to be mediated by direct activation of somatodendritic autoreceptors of the 5-HT$_{1A}$ subtype (Sotelo et al., 1990), which cause opening of K$^+$ channels via a pertussis toxin-sensitive G protein and, consequently, membrane hyperpolarization (Innis et al., 1988; Williams et al., 1988). The dorsal raphe electrophysiological assay also has proved to be a very sensitive indicator of partial agonist activity at 5-HT$_{1A}$ receptors because the large autoreceptor reserve of dorsal raphe neurons (Cox et al., 1993) has permitted detection of weak agonist properties even among drugs that appeared in other assays to act exclusively as antagonists. Although the inhibition of dorsal raphe neuron firing by their own transmitter is a critical autoregulatory mechanism, it is important to note that these cells also are subject to a tonic excitatory noradrenergic input (Baraban and Aghajanian, 1981), and blockade of this influence by alpha-1 adrenergic antagonists represents a second pharmacological means of suppressing dorsal raphe cell firing (Baraban and Aghajanian, 1980; Marwaha and Aghajanian, 1982).

Because there have been no comparative evaluations of the new group of putative 5-HT$_{1A}$ antagonists on dorsal raphe neuronal activity, we assessed the antagonist as well as possible agonist-like properties of these drugs in this system by (1) determining their abilities to elicit rightward shifts in the dose-response curve of dorsal raphe neurons to i.v. 8-OH-DPAT, (2) constructing i.v. dose-response curves for their abilities to inhibit dorsal raphe cell firing over an extensive range of doses (0.001–10 μmol/kg), and (3) determining whether they could reverse the inhibition of raphe cell firing elicited by 8-OH-DPAT. The following putative 5-HT$_{1A}$ receptor antagonists were evaluated: WAY 100635 ([N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyrindinyl) cyclohexane-carboxamide; Forster et al., 1995; Fletcher et al., 1996; Fornal et al., 1996], p-MPPI [4-(2-methoxyphenyl)-1-[2’-[N-(2’-pyrindinyl)-p-iodobenzamido]ethyl]piperazine; Kung et al., 1994; Thielen et al., 1996], and two new compounds synthesized and developed by Astra Arcus (Sweden), NAD-299 [(R)-3-N,N-dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide; Johansson et al., 1997] and NDL-249 [(R)-3-(N-propylamino)-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide].

**Materials and Methods**

**Extracellular Single-Unit Recording Techniques.** Male Sprague-Dawley rats (Taconic, NY) weighing between 250 and 350 g were anesthetized with chloral hydrate (400 mg/kg i.p.) and positioned in a stereotaxic apparatus. Body temperature was monitored by a rectal probe and maintained between 36–38°C with a feedback-controlled heating pad. A lateral tail vein was cannulated to allow i.v. administration of additional chloral hydrate, as well as the test compounds. The scalp was retracted, and a small burr hole was drilled through the skull overlying the dorsal raphe nucleus. The dura was removed with care taken to not lacerate the sagittal sinus, and any meningeal bleeding was controlled with a hemostatic gel. Neurons of the dorsal raphe were located within the stereotaxic boundaries: 0.0 to 0.2 mm lateral to midline, 0.3 to 0.7 mm anterior to the lambdoid suture, and 5.5 to 6.5 mm ventral to the surface of the brain. These cells were identified by their electrophysiological characteristics as previously described (Rogawski and Aghajanian, 1981; Sprouse and Aghajanian, 1987; Cox et al., 1993). Briefly, dorsal raphe serotonergic cells characteristically demonstrated a slow firing rate (0.2–3 spikes/s) and a wide, positive-negative extracellular action potential of approximately 0.9- to 1.2-ms duration.

Standard extracellular single-unit recording methods were used to monitor the activity of spontaneously active dorsal raphe neurons (Rogawski and Aghajanian, 1981; VanderMaelen et al., 1986; Sprouse and Aghajanian, 1987). Single-barrel glass microelectrodes were prepared from 2.0 mm (o.d.) capillary tubing and filled with 2 M sodium chloride solution containing 1% Pontamine Sky Blue dye. After filling, electrode tips were broken back under light microscopy to 1 to 2 μm corresponding to an in vitro impedance of 3 to 10 MΩ measured at 135 Hz. Extracellular action potentials of raphe neurons were amplified, filtered, and displayed on an oscilloscope screen and passed to a window discriminator and rate meter. Impulse rates were summed over 10-s intervals and simultaneously printed by a digital printer. Firing rates were displayed as histogram plots of spikes/10 s versus time.

Only one cell was recorded from each rat to avoid residual drug effects. At the end of each experiment, blue dye was iontophoresetically ejected from the electrode tip for 20 to 30 min. Brains were fixed, and then sectioned and examined, to confirm that the blue dye deposit marking the recording site was within the dorsal raphe nucleus.

Responses to the drugs were quantified by comparing the average firing rate during the 60-s period after each dose with the mean baseline firing rate for that neuron. The responses of 7 to 14 cells were averaged for each drug at each dose, and mean responses ± S.E.M. were plotted as a function of the log dose. ED$_{50}$ values were derived from best-fit sigmoidal curves generated by a nonlinear curve-fitting computer program (GraphPAD Software, San Diego, CA), which fitted the curve to the mean firing rate at each dose.

**Intravenous Dose-Response Curves.** After encountering a spontaneously active neuron with electrophysiological characteristics of a serotonergic cell, a 3- to 5-min period of stable baseline firing was recorded. Dose-response curves to 8-OH-DPAT, WAY 100635, NDL-249, NAD-299, and p-MPPI were constructed by injecting doses i.v. at 1-min intervals so that each successive dose doubled the previous cumulative dose. The dose range for the suppression of raphe cell firing by 8-OH-DPAT was established in our earlier study (Cox et al., 1993). Dose ranges for WAY 100635, NDL-249, NAD-299, and p-MPPI were selected on the basis of prior in vivo behavioral and/or electrophysiological studies in rats, as reported previously (Forster et al., 1995; Thielen and Frazer, 1995; Fletcher et al., 1996; Thielen et al., 1996; Johansson et al., 1997). Initial doses were 0.32 μg/kg (0.001 μmol/kg) for 8-OH-DPAT and 1 μg/kg for WAY 100635 (0.0018 μmol/kg), NDL-249 (0.0028 μmol/kg), NAD-299 (0.0021 μmol/kg), and p-MPPI (0.0017 μmol/kg). Additional doses were administered until spontaneous firing was completely inhibited or a cumulative dose of >4096 μg/kg was given. This logarithmic sequence of drug administration at 1-min intervals is conventional in...
in vivo electrophysiological studies of monoaminergic neurons when comparing the actions of a series of structurally related, lipophilic drugs that readily cross the blood-brain barrier (Martin et al., 1990; Cox et al., 1993). In using this paradigm, it is recognized that differences in the pharmacokinetics of brain uptake may contribute to differences in the observed magnitude of drug effects.

In another series of experiments, the antagonist properties of the drugs were assessed by evaluating their abilities to shift rightward the dose-response curve to 8-OH-DPAT. Dose-response curves to 8-OH-DPAT were initiated 3 to 5 min after i.v. administration of roughly equimolar doses (0.02–0.03 \mu g/kg) of either WAY 100635 (10 \mu g/kg; 0.02 \mu mol/kg), NDL-249 (10 \mu g/kg; 0.03 \mu mol/kg), p-MPPI (10 \mu g/kg; 0.02 \mu mol/kg), or NAD-299 (13.6 \mu g/kg; 0.03 \mu mol/kg). After achieving a full inhibition of raphe cell firing with 8-OH-DPAT, additional doses of the putative antagonists were given to determine whether the inhibition could be reversed.

Attempts to Reverse Inhibitions of Raphe Cell Firing with \textit{d}-Amphetamine. To evaluate whether inhibitory effects of the above drugs might be mediated by \textit{alpha} adrenergic receptor blockade, an attempt was made to reverse the inhibition of firing by subsequent i.v. administration of \textit{d}-amphetamine (3.2 mg/kg). If the depressant effects of these compounds were due to adrenergic receptor blockade, then \textit{d}-amphetamine (which induces release of norepinephrine) might be expected to reverse the inhibitory effect.

Materials. 8-OH-DPAT was obtained from Research Biochemicals International (Natick, MA), WAY 100635, NDL-249, and NAD-299 were all synthesized and provided by Astra Arcus (Södertälje, Sweden). p-MPPI was generously supplied by Dr. Hank Kung (University of Pennsylvania, Philadelphia, PA). Solutions of the above drugs were prepared fresh before use in 0.9% NaCl at a concentration of 0.25 or 1.0 mg/ml. Chloral hydrate (Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water.

Results

Antagonist Properties of WAY 100635, NAD-299, NDL-249, and p-MPPI. In agreement with previous reports, 8-OH-DPAT was found to be an extremely potent agonist in inhibiting the firing of dorsal raphe neurons (Figs. 1 and 2). In the present studies, 8-OH-DPAT caused a complete suppression of raphe cell firing at doses below 10 \mu g/kg and with a calculated ED_{50} of 1.3 ± 0.3 \mu g/kg. Prior administration of each of the four putative 5-HT_{1A} receptor antagonists, WAY 100635, NDL-249, NAD-299, or p-MPPI (doses equivalent to 0.02–0.03 \mu mol/kg), produced significant rightward shifts in the dose-response curve of dorsal raphe neurons to 8-OH-DPAT (Figs. 1 and 2). The ED_{50} values for 8-OH-DPAT alone, and after pretreatment with each of the antagonists, are given in Table 1. These findings suggest that WAY 100635, NAD-299, NDL-249, and p-MPPI can act as antagonists of the effects of 8-OH-DPAT at 5-HT_{1A} somatodendritic autoreceptors.

Additionally, in all cases where attempted, subsequent administration of WAY 100635, NAD-299, and NDL-249 (10–20 \mu g/kg) was able to reverse the inhibition of cell firing elicited by i.v. administration of 8-OH-DPAT (Fig. 1). In contrast, however, p-MPPI (10–20 \mu g/kg) did not reverse inhibitions by 8-OH-DPAT in five of six cases where attempted.

Effects of WAY 100635, NAD-299, NDL-249, and p-MPPI on Raphe Cell Firing. To assess whether the above drugs might possess agonist-like properties, changes in raphe cell firing were monitored during i.v. administration of each of the putative antagonists over an extensive range of doses. Over the lower range of doses tested, none of the drugs caused changes in firing exceeding ±20% of the basal firing rate before drug injection. Unexpectedly, at higher doses, the administration of WAY 100635, NAD-299, and NDL-249 each produced dose-dependent inhibitions of dorsal raphe cell firing (Figs. 3 and 4). Both WAY 100635 and NDL-249 caused complete inhibitions of firing, whereas NAD-299 caused somewhat less than a full inhibition in some cells. However, the suppression of firing by these three compounds was observed only at high doses (>0.1 \mu mol/kg), whereas 8-OH-DPAT inhibited firing at doses 1 to 2 orders of magnitude lower. In contrast, p-MPPI caused
no consistent inhibitory effects at similarly high (i.e., equimolar) doses; some raphe cells were moderately inhibited (see Fig. 3), whereas others exhibited large fluctuations in firing and/or appeared unstable and stimulated at the higher range of doses (Fig. 4).

We also found that lower doses of WAY 100635 sometimes caused a modest increase in firing and that average firing rates did not fall significantly below baseline until the administration of a cumulative dose of 128 \( \mu \text{g/kg} \) (0.23 \( \mu \text{mol/kg} \)). Increases in firing were not observed at the low range of doses of NDL-249 or NAD-299. The cumulative dose-response curves for the effects of these drugs on raphe cell firing are shown in Fig. 4, and their average ED\text{50} values for the inhibition of raphe cell firing are given in Table 2.

Reversal by \( d \)-Amphetamine of Effects of WAY 100635, NAD-29, and NDL-249. Because the firing of dorsal raphe neurons can also be inhibited by administration of \( \alpha \)-adrenergic antagonists that block the tonic excitatory noradrenergic input these cells receive (Baraban and Aghajanian, 1980; Marwaha and Aghajanian, 1982), it was of interest to determine whether the inhibitory effects of WAY 100635, NAD-299, and NDL-249 were due to \( \alpha \)-adrenergic receptor blockade rather than to 5-HT\text{1A} receptor agonism. If \( \alpha \)-receptor blockade were responsible, the effect should be reversed by increasing adrenergic tone. In accordance with this prediction, the inhibition caused by each of the three putative antagonists could be rapidly and fully reversed by a moderate (3.2 \( \mu \text{g/kg} \)) i.v. dose of \( d \)-amphetamine (\( n = 3–4 \) for each antagonist), whereas the inhibition elicited by the selective 5-HT\text{1A} receptor agonist 8-OH-DPAT was not reversed by \( d \)-amphetamine, even after administration of doses up to 6.4 or 12.8 \( \mu \text{g/kg} \) (\( n = 5 \); Fig. 5).

**Discussion**

The present study represents the first comparative evaluation of a series of “silent” 5-HT\text{1A} receptor antagonists in a sensitive in vivo assay of 5-HT\text{1A} somatodendritic autoreceptor activity. We evaluated and compared the antagonist potency as well as the intrinsic effects of the two most widely cited silent 5-HT\text{1A} receptor antagonists, WAY 100635 and

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**TABLE 1**

<table>
<thead>
<tr>
<th>Pretreatment Drug</th>
<th>8-OH-DPAT ED\text{50} ( \mu \text{g/kg} )</th>
<th>Fold Shift</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.3 ± 0.3</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>WAY 100635</td>
<td>18.9 ± 2.9(^*)</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>NDL-249</td>
<td>6.0 ± 1.2(^*)</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>NAD-299</td>
<td>18.2 ± 1.0(^*)</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>( p )-MPPI</td>
<td>4.7 ± 1.1(^*)</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

Value significantly different from no pretreatment, \(^*\) \( p < .001 \).
Firing elicited by a single i.v. dose of WAY 100635 (20 mg/kg) to readily and fully reverse the inhibition of rat dorsal raphe cell firing.

**TABLE 2**

Comparison of ED50 values for the inhibition of dorsal raphe cell firing by 8-OH-DPAT, WAY 100635, NDL-249, NAD-299, and p-MPPI.

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED50 µg/kg</th>
<th>ED50 µmol/kg</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OH-DPAT</td>
<td>1.3 ± 0.3</td>
<td>0.004 ± 0.0009</td>
<td>14</td>
</tr>
<tr>
<td>WAY 100635</td>
<td>330 ± 110</td>
<td>0.6 ± 0.2</td>
<td>5</td>
</tr>
<tr>
<td>NDL-249</td>
<td>250 ± 107</td>
<td>0.7 ± 0.3**</td>
<td>7</td>
</tr>
<tr>
<td>NAD-299</td>
<td>435 ± 216</td>
<td>0.9 ± 0.4***</td>
<td>6</td>
</tr>
<tr>
<td>p-MPPI</td>
<td></td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

Value significantly different from 8-OH-DPAT: *p < .001; **p < .003; ***p < .002.

Another outcome of these experiments was the first demonstration of antagonist activity of p-MPPI in the dorsal raphe electrophysiological assay. Previous reports have shown that p-MPPI behaves as an antagonist in both in vitro and in vivo tests of postsynaptic 5-HT1A receptor activity, such as blockade of the 8-OH-DPAT-induced inhibition of forskolin-stimulated adenylyl cyclase in rat hippocampal membranes (Kung et al., 1994) and blockade of 8-OH-DPAT-induced hypothermia and reciprocal forepaw treading (Thielen and Frazer, 1995; Thielen et al., 1996). To date, p-MPPI has been evaluated in only one assay of in vivo somatodendritic autoreceptor activity (i.e., changes in the 5-hydroxyindole acetic acid/5-HT ratio in the rat hippocampus and striatum), and it was shown to inhibit the 8-OH-DPAT-induced decrease in 5-hydroxyindole acetic acid/5-HT in both brain areas. However, in this assay, a comparatively high (5–10 mg/kg) i.p. dose was required to block the effect of 8-OH-DPAT (Thielen et al., 1996). In our studies, an i.v. dose of p-MPPI that was 1000-fold lower (10 µg/kg) produced a significant 4-fold rightward shift in the dose-response curve for the inhibition of raphe cell firing by 8-OH-DPAT, confirming the antagonist action of p-MPPI in this more sensitive measure of somatodendritic autoreceptor activity. It is not clear why subsequent administration of an additional 10 to 20 µg/kg dose of p-MPPI was unable to reverse the inhibition of raphe cell by 8-OH-DPAT, as was possible with the other compounds. Pharmacokinetic factors, such as a slower brain uptake, or the somewhat lower affinity of p-MPPI at 5-HT1A receptors (Ki = 0.2, 0.6, and 1 nM for WAY 100635, NAD-299, and p-MPPI, respectively; Kung et al., 1994; Johansson et al., 1997) might account for its inability to reverse the effect of 8-OH-DPAT. Higher doses may need to have been given to demonstrate reversal with p-MPPI.

Our results also provide the first published evidence of 5-HT1A somatodendritic autoreceptor antagonist activity of two newer compounds, NAD-299 and NDL-249, in the dorsal raphe electrophysiological assay and show that the former drug causes a shift in the 8-OH-DPAT dose-response curve similar in magnitude to that of a roughly equimolar dose of
WAY 100635. This finding places NAD-299 on a par with WAY 100635 as one of the two most potent 5-HT$_{1A}$ antagonists yet described in this test system. The similarity in the degree of shift in the 8-OH-DPAT dose-response curves by NAD-299 and WAY 100635 in our study contrasts with the somewhat higher potency of WAY 100635 for antagonizing various other 8-OH-DPAT-induced responses in vivo and in vitro (Johansson et al., 1997). The apparent difference in potency between our study and other in vivo assays may reflect differences in routes of administration and pharmacokinetics rather than true differences in antagonist activity of the two compounds. In each of these tests, neither WAY 100635 nor NAD-299 exhibited any intrinsic agonist activity by themselves (Johansson et al., 1997).

A second line of experiments examined whether the four antagonists might possess intrinsic effects on dorsal raphe cell firing when administered alone over an extensive range of doses. Unexpectedly, three of the four compounds, WAY 100635, NDL-249, and NAD-299, also caused dose-dependent and ultimately complete inhibitions of raphe cell firing. However, inhibitory effects were typically observed only at very high doses (above 0.1 $\mu$mol/kg) of each drug. The ED$_{50}$ values for inhibition were 30-fold higher than the doses that were shown in our earlier study to antagonize the actions of 8-OH-DPAT on raphe cell firing. Nevertheless, the inhibitory effects contrast with previous reports on WAY 100635 that have asserted that this drug lacks agonist-like effects in a broad range of tests of either postsynaptic or somatodendritic 5-HT$_{1A}$ receptor activity (Forster et al., 1995; Corradetti et al., 1996; Fletcher et al., 1996; Fornal et al., 1996). It should be noted that in previous studies performed in anesthetized rats, which most closely parallel our studies, effects of i.v. administration of WAY 100635 on raphe cell firing were evaluated only to a maximal dose of 100 $\mu$g/kg (Forster et al., 1995; Fletcher et al., 1996), a dose on the threshold of inhibition of firing in our studies. In awake cats, doses as high as 500 $\mu$g/kg, somewhat higher than the ED$_{50}$ for inhibition in rats, caused only increases in raphe cell firing (Fornal et al., 1996). In view of the species difference and the fact that anesthesia may alter autoreceptor modulation of raphe neuronal activity (Fornal et al., 1996a), it is unclear whether doses higher than 500 $\mu$g/kg would have suppressed raphe cell firing in cats. Nevertheless, our results do suggest that inhibitory effects of WAY 100635 on raphe cell firing were not detected in earlier studies on rats because the doses given were not high enough. They further suggest that raphe cell firing may be partially to fully inhibited in vivo models where WAY 100635 is given to rats in doses ranging from 0.1 to 1 mg/kg and that this inhibition of serotonergic neuronal activity might contribute to the observed biochemical and behavioral effects. Likewise, similarly high doses of NAD-299 or NDL-249 might be expected to suppress raphe cell firing in vivo studies in rats, although the inhibitory effects of these drugs may not be mediated by an action at 5-HT$_{1A}$ receptors (see discussion below).

Unlike the responses to WAY 100635, NAD-299, and NDL-249, no consistent effects on raphe cell firing were observed with p-MPPI. There was, however, considerable variability in response to this drug, with some cells showing increases as well as moderate decreases in firing. At the higher range of doses, recordings frequently became unstable with broadening of the extracellular spike and a shift to a burst pattern of firing. Overall, the lack of change in the average firing rates distinguishes p-MPPI from the other drugs tested. The basis for this difference in response is not clear. It is conceivable that nonspecific (i.e., nonpharmacological) membrane-depolarizing effects of p-MPPI masked a tendency of this drug to inhibit raphe cell firing, at least for some of the cells tested.

The inhibitory effects of the other three compounds raise questions about the mechanism underlying this effect. Considering the fact that numerous drugs (including BMY 7378, ipsapirone, buspirone, NAN-190, and WAY 100135) were previously claimed to be 5-HT$_{1A}$ receptor antagonists but later found to behave as agonists in the dorsal raphe system (Claustre et al., 1991; Cox et al., 1993, Fornal et al., 1994a, b, 1996), we considered the possibility that these 5-HT$_{1A}$ receptor antagonists might also possess weak 5-HT$_{1A}$ receptor agonist activity. Although previous studies with both WAY 100635 and NAD-299, including biochemical and electrophysiological assays of autoreceptor function (Fletcher et al., 1996; Johansson et al., 1997; Ahlenius et al., 1998), have detected no evidence of intrinsic activity at 5-HT$_{1A}$ receptors, the inhibition of dorsal raphe cell firing might represent a more sensitive assay for detecting weak agonist efficacy. Alternatively, because extremely high doses were required to elicit the response, it was conceivable that the inhibition of firing was mediated via another receptor type, such as alpha adrenergic receptor blockade. Alpha adrenergic receptor antagonists have long been known to produce effects similar to 5-HT$_{1A}$ receptor agonists (i.e., they inhibit the firing of dorsal raphe neurons; Baraban and Aghajanian, 1980; Marwaha and Aghajanian, 1982) but in this case by blocking the tonic noradrenergic excitatory influence these cell receive (Baraban and Aghajanian, 1981). Accordingly, alpha adrenergic agonists and drugs that increase adrenergic tone, such as amphetamine, have been shown to reverse this effect and restore firing (Baraban and Aghajanian, 1980). We used a similar strategy to evaluate whether alpha adrenergic receptor blockade, rather than 5-HT$_{1A}$ receptor agonism, might mediate the inhibition of raphe cell firing in our studies and found that i.v. administration of a 3.2 mg/kg dose of d-amphetamine could indeed readily and fully reverse the inhibitory effects of high doses of WAY 100635, NAD-299, and NDL-249, although the inhibition by the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT was not antagonized. Similar in vivo doses of amphetamine (2.5–3.0 mg/kg) have been shown to cause a rapid and pronounced increase in extracellular norepinephrine in rat cortex and hippocampus (Kuczenski and Segal, 1992) but no significant change in extracellular serotonin levels (Kankaanpää et al., 1998). Thus, the ability of amphetamine to reverse the inhibitory effects of the three 5-HT$_{1A}$ receptor antagonists is most likely a consequence of elevated norepinephrine levels in the raphe and the subsequent displacement of these antagonists from alpha adrenergic receptors.

In further support of alpha adrenergic receptor blockade as a possible mechanism for the rate-suppressant effects, each of the drugs we evaluated binds to alpha-1 adrenoceptors at mid to high nanomolar concentrations. For instance, $K_v$ values for WAY 100635, p-MPPI, and NAD-299 at alpha-1 receptors are 45, 35, and 260 nM, respectively (Kung et al., 1994; Johansson et al., 1997); a $K_v$ of 270 nM has been determined for NDL-249 (L. Unelius and N. Mohell, personal...
communication). Although the affinity for 5-HT<sub>1A</sub> receptors exceeds that of alpha-1 receptors by almost 200-fold for WAY 100635 and by more than 400-fold for NAD-299 (Johansson et al., 1997), the concentrations achieved in brain after >0.1 μmol/kg i.v. doses of these drugs may have exceeded the concentration range over which they are selective for 5-HT<sub>1A</sub> receptors. In support of this possibility, Craven et al. (1994) reported that a high (300 nM) concentration of WAY 100635 caused a gradual but pronounced slowing of rat cell firing in a slice preparation of the guinea pig dorsal raphe nucleus, and they attributed the effect to antagonism of the alpha-1 receptor agonist phenylephrine, which was added to stimulate raphe cell firing in the slice. Thus, our finding that the in vivo inhibition of raphe cell firing by high doses of WAY 100635, NAD-299, and NDL-249 could be reversed by d-amphetamine suggests that the inhibitory effects were likely to have been mediated by alpha adrenergic receptor blockade rather than 5-HT<sub>1A</sub> receptor antagonism. However, a contribution by the latter or other mechanisms cannot be definitively ruled out on this basis.

In summary, we confirmed the antagonist properties of WAY 100635 and p-MPPI in an assay of somatodendritic 5-HT<sub>1A</sub> autoreceptor activity, and we provided evidence that two newer compounds, NAD-299 and NDL-249, possess a similar profile as 5-HT<sub>1A</sub> antagonists in this system. Indeed, NAD-299 exhibits antagonist potency in this system on a par with the reference antagonist at 5-HT<sub>1A</sub> receptors, WAY 100635. Although very high (>0.1 μmol/kg) doses of WAY 100635, NAD-299, and NDL-249 can also inhibit raphe cell firing, this effect appears not to be attributable to 5-HT<sub>1A</sub> receptor partial agonist activity but may instead be due to alpha-1 adrenergic receptor blockade. Thus, we conclude that the four drugs behave as pure or “silent” antagonists at the 5-HT<sub>1A</sub> autoreceptors regulating dorsal raphe neuronal firing and that at appropriate doses (below 0.1 μmol/kg in vivo), these drugs may serve as useful tools for distinguishing specifically the functions of 5-HT<sub>1A</sub> receptors.

References


