Dextromethorphan and Its Metabolite Dextrorphan Block α3β4 Neuronal Nicotinic Receptors1,2

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ABSTRACT

Dextromethorphan (DM), a structural analog of morphine and codeine, has been widely used as a cough suppressant for more than 40 years. DM is not itself a potent analgesic, but it has been reported to enhance analgesia produced by morphine and nonsteroidal anti-inflammatory drugs. Although DM is considered to be nonaddictive, it has been reported to reduce morphine tolerance in rats and to be useful in helping addicted subjects to withdraw from heroin. Here we studied the effects of DM on neuronal nicotinic receptors stably expressed in human embryonic kidney cells. Studies were carried out to examine the effects of DM on nicotine-stimulated whole cell currents and nicotine-stimulated 46Rb+ efflux. We found that both DM and its metabolite dextrorphan block nicotinic receptor function in a noncompetitive but reversible manner, suggesting that both drugs block the receptor channel. Consistent with blockade of the receptor channel, neither drug competed for the nicotinic agonist binding sites labeled by [3H]epibatidine. Although DM is approximately 9-fold less potent than the widely used noncompetitive nicotinic antagonist mecamylamine in blocking nicotinic receptor function, the block by DM appears to reverse more slowly than that by mecamylamine. These data indicate that DM is a useful antagonist for studying nicotinic receptor function and suggest that it might prove to be a clinically useful neuronal nicotinic receptor antagonist, possibly helpful as an aid for helping people addicted to nicotine to refrain from smoking, as well as in other conditions where blockade of neuronal nicotinic receptors would be helpful.

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Dextromethorphan (DM) is structurally closely related to levorphanol, codeine, and morphine, but unlike these opiates it has low affinity for opiate receptors and is not considered to be addictive. Nevertheless, it shares with most opiates the ability to suppress cough and has been used as an effective antitussive drug for more than 40 years. DM appears to produce little analgesia by itself, it has recently been shown to attenuate tolerance and/or enhance analgesia produced by morphine (Elliott et al., 1994; Mao et al., 1996) and nonsteroidal anti-inflammatory drugs (Price et al., 1996). Furthermore, DM has been reported to reduce morphine dependence in rats (Mao et al., 1996) and possibly to be useful in treating addicted subjects withdrawing from heroin (Koyuncuoglu and Saydam, 1990). In addition, DM has been shown to have neuroprotective effects in models of glutamate neurotoxicity (Choi, 1987; Choi et al., 1987).

Some of these diverse effects may be related to the ability of DM and/or its demethylated major metabolite dextrophan (DP) to block N-methyl-D-aspartate (NMDA) receptor channels (Church et al., 1985); however, there is one report that indicates that DM might also block nicotinic receptors in PC12 cells (Yamamoto et al., 1992). We have recently stably expressed and characterized a neuronal nicotinic receptor comprised of α3 and β4 subunits in HEK-293 cells (Xiao et al., 1998). This stably transfected cell line, designated KXo3β4R2, expresses a high density of nicotinic receptors that can be labeled by [3H]epibatidine ([3H]EB) and that pass 46Rb+ in response to stimulation by the agonists acetylcholine, nicotine, cytisine, and epibatidine. The function of these receptors is blocked by the nicotinic antagonists mecamylamine, d-tubocurarine, hexamethonium, and dihydro-β-erythroidine (Xiao et al., 1998). These cells provide an excellent model system in which to examine drug effects on the α3β4 nicotinic receptor. Here, we have examined and compared the effects of DM and DP, their structural analogs, and several other drugs that block nicotinic receptor and/or NMDA receptor channels, on nicotine-stimulated receptor function in these cells.

Experimental Procedures

Materials and Drugs. Tissue culture medium, serum, and antibiotics were purchased from Life Technologies (Gaithersburg, MD).

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[3H]EB and 86RbCl were supplied by NEN (Boston, MA). Chemicals were purchased from Fisher Scientific Co. (Fairlawn, NJ). (-)-Nicotine, DM, DP, and mecamylamine were purchased from Sigma Chemical Co. (St. Louis, MO). MK-801 was purchased from Research Biochemicals International (Natick, MA), and Phencyclidine was provided by the National Institute on Drug Abuse. All other chemicals were reagent grade.

**Cell Culture.** KXo3B4 cells were grown as described previously (Xiao et al., 1998) in minimum essential medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 0.7 mg/ml of gentamicin. Cells were maintained at 37°C with 5% CO₂ in a humidified incubator.

**Ligand Binding Assay.** The function of nicotinic acetylcholine receptors expressed in the KXo3B4 cells was measured using [3H]EB ligand binding assay (Lukas and Cullen, 1988; Xiao et al., 1998). Briefly, 1-ml aliquots of cells in growth medium were plated on 24-well plates coated with poly(A)-lysine). The plated cells were grown at 37°C for 16 to 18 h until reaching 90 to 100% confluence. On the day of the experiment, the growth medium was aspirated and the cells were incubated in fresh medium containing 2 mM [3H]EB, which binds with high affinity to the αβ4 nicotinic receptors.

**Radioligand Binding Assay.** Nicotinic receptor binding sites in membrane homogenates from KXo3B4 cells were measured using [3H]EB, which binds with high affinity to the αβ4 receptors (Xiao et al., 1998). DM or DP at concentrations from 1 to 100 μM competed against 300 pM [3H]EB for receptors in KXo3B4 cell membranes. Ligand binding was measured as described previously (Xiao et al., 1998). Briefly, cultured cells at >90% confluence were harvested in 1 ml of medium, and nicotinic-stimulated 86Rb⁺ efflux was measured as above.

**Electrophysiology.** Ionic currents in whole-cell configuration were measured using the patch-clamp technique and a fast drug delivery system. Cells were maintained at a holding potential of approximately 60 mV. Briefly, cells coated onto coverslips were positioned in a recording chamber (1-ml volume) and perfused with Ringer’s solution (120 mM NaCl, 3.1 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 26 mM NaHCO₃, 1 mM K₂HPO₄, and 5 mM glucose) at 24°C. The solution was continuously bubbled with 5% CO₂ and 95% O₂ to maintain the pH at 7.4. Cells were visually identified using an upright microscope (Axioskop; Carl Zeiss, Jena, Germany). A gravity-fed Y-tubing system was placed within 100 μm of the cell under investigation, and a complete change of solution surrounding the cell was achieved in less than 1 s. The pipette solution contained 145 mM CsCl, 1 mM MgCl₂, 2 mM ATP, 1 mM EGTA, and 10 mM HEPES (pH was adjusted to 7.2 with CsOH).

**Results**

**Effects of DM and DP on Nicotinic Receptors.** The effects of DM and its metabolite DP on αβ4 nicotinic receptors were examined first in assays that measure nicotine-stimulated 86Rb⁺ efflux through the nicotinic receptor channel. In these cells, nicotine stimulates 86Rb⁺ efflux with an EC₅₀ of ~28 μM, and 100 μM nicotine stimulates a nearly maximal 86Rb⁺ efflux response, which is approximately 8 times basal efflux (Xiao et al., 1998). Both DM and DP blocked this receptor function stimulated by 100 μM nicotine in a concentration-related manner, with IC₅₀ values of approximately 9 and 30 μM, respectively (Fig. 1 and Table 1).

![Fig. 1. Inhibition by DM and DP of nicotine-stimulated 86Rb⁺ efflux from KXo3B4 cells.](https://example.com/fig1.png)
We also examined the effect of DM on nicotinic receptors in whole-cell patch-clamp studies. Nicotine stimulates a large inward current in these cells (Fig. 2a). Consistent with its effect on nicotine-stimulated $^{86}$Rb⁺ efflux, DM at a concentration of 100 μM nearly completely blocked the nicotine-activated current (Fig. 2b). After an 8-min drug washout period, a substantial fraction of the nicotine-stimulated receptor function returned (Fig. 2c). However, the function had not fully recovered compared with the initial response to nicotine (compare Fig. 2, a and c), suggesting either that some residual DM remained in the preparation, or that the receptor was partially desensitized at this time after the prior exposure to nicotine.

**Mode of Nicotinic Receptor Block by DM and DP.** To determine the type of pharmacological block produced by DM and DP, the effects of these drugs on the concentration-response relationship for nicotine-stimulated $^{86}$Rb⁺ efflux were examined. As shown in Fig. 3a, at concentrations near their IC₅₀ values both DM and DP decreased the maximum nicotine-stimulated response without significantly shifting the EC₅₀ for nicotine in the concentration-response curves. This result is consistent with a noncompetitive pharmacological block and suggests that DM and DP block the nicotinic receptor channel without necessarily binding to the agonist recognition site. To assess this further, we examined the ability of these drugs to compete for α3β4 receptor agonist binding sites labeled by $[^3H]$EB in membrane homogenates from these cells. Neither DM nor DP competed effectively for

**TABLE 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC₅₀ (μM)</th>
<th>n_H</th>
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<tbody>
<tr>
<td>DM</td>
<td>8.9 ± 1.1</td>
<td>1.2 ± 0.08</td>
</tr>
<tr>
<td>DP</td>
<td>29.6 ± 5.7</td>
<td>1.0 ± 0.05</td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>1.0 ± 0.04</td>
<td>1.2 ± 0.15</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>7.0 ± 1.3</td>
<td>1.2 ± 0.09</td>
</tr>
<tr>
<td>MK-801</td>
<td>26.6 ± 9.6</td>
<td>1.1 ± 0.19</td>
</tr>
</tbody>
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$^{86}$Rb⁺ efflux was measured as described in Experimental Procedures. IC₅₀ values and Hill coefficients (n_H) were calculated from the Hill equation. Values shown are the mean ± S.E. of three experiments.

![Fig. 2. Inhibition by DM of nicotine-stimulated whole-cell currents in KXα3β4 cells.](image)

![Fig. 3. Mechanism of nicotinic receptor inhibition by DM and DP.](image)
the α3β4 receptor agonist binding sites labeled by [3H]EB; for example, at a concentration of 100 μM, DM and DP inhibited [3H]EB binding by no more than 20% (Fig. 3b).

**Recovery of Receptor Function after Exposure to DM or mecamylamine.** To examine the reversibility and time course of recovery of receptor function after exposure to DM, cells were exposed to 10 μM DM for 30 min or for 24 h before being prepared for measurement of nicotine-stimulated 86Rb⁺ efflux. The preparation of the cells after drug exposure included four washes with HEPES buffer over a 7-min period, including one 5-min wash period, to effectively remove any drug free in solution. Despite this washing procedure, the maximum nicotine-stimulated 86Rb⁺ efflux was still decreased by approximately 40 and 60% in cells exposed to DM for 30 min and 24 h, respectively (Fig. 4a). For comparison, the effect of mecamylamine, a well established nicotinic receptor channel blocker, was measured in parallel under the same conditions. Mecamylamine is more potent than DM at blocking the α3β4 receptors in these cells (see Table 1), but in contrast to the effects of DM, the 7-min washing procedure nearly completely reversed the effects of both the 30-min and the 24-h exposure to 10 μM mecamylamine (Fig. 4a).

To determine the extent to which a longer time of exposure to DM would affect receptor function and whether the receptor function would fully recover after longer periods free of drug, cells in culture were exposed to 10 μM DM for 4 or 5 days. The drug was then removed by washing and the cells were allowed to recover in media in the absence of drug for a total time of either 7 min, 2 h, or 1 day before nicotine-stimulated 86Rb⁺ efflux was measured. As shown in Fig. 4b, after exposure to 10 μM DM for 5 days followed by a routine 7-min washing procedure, as described above, the maximum nicotine-stimulated 86Rb⁺ efflux was still decreased by about 45%, but in cells allowed to recover in the absence of DM for 2 h or 1 day, maximum nicotine-stimulated 86Rb⁺ efflux was fully restored. Thus, under these conditions, the blockade of α3β4 receptor function by DM is fully reversible within 2 h after removal of the drug.

**Comparison of DM and DP to Structural Analogs of Opiates and Other Receptor Channel Blockers.** Both DM and DP are structural analogs of opiates; therefore, we measured the nicotinic receptor blocking activity of two other opiates, codeine and hydrocodone, both of which retain the methoxy group at the 3 position and the N-methyl group at position 17 of the more potent DM. Although both opiates appeared to be capable of blocking nicotine-stimulated 86Rb⁺ efflux, they were much less potent than DM or DP; at a concentration of 100 μM, codeine and hydrocodone blocked efflux by less than 20 and 30%, respectively (Fig. 5).

Finally, because DM and DP are, in addition to opiate receptor agonists, NMDA receptor antagonists (Church et al., 1985), we compared them to several known nicotinic and/or NMDA receptor channel blockers. The potency of the drugs tested to block these nicotinic receptors appeared to be divided into three groups (Fig. 6; Table 1). Mecamylamine was the most potent drug, followed by DM and phencyclidine, which were nearly equipotent to each other; finally, MK-801, which is a potent NMDA receptor channel blocker, and DP were the least potent among these drugs in blocking the nicotinic receptor-mediated response. All of the drugs tested...
here blocked the receptor with a Hill coefficient close to 1 (Table 1).

Discussion

The studies presented here demonstrate that both DM and its metabolite DP block \( \alpha_3\beta_4 \) nicotinic receptors noncompetitively. The noncompetitive nature of the blockade and the observation that neither DM nor DP competes for the agonist binding site strongly suggest that these drugs bind within and block the receptor channel. DM, in particular, is a relatively potent nicotinic receptor blocker, with an IC\(_{50}\) of less than 10 \( \mu \)M in assays that measured \(^{86}\)Rb\(^{+}\) efflux stimulated by 100 \( \mu \)M nicotine; consistent with this potency, DM nearly completely blocked whole cell currents stimulated by 10 \( \mu \)M nicotine. The apparent potency of DM at \( \alpha_3\beta_4 \) receptors is similar to that of phencyclidine and about 9-fold lower than that of mecamylamine, which is one of the most potent blockers of these receptors reported so far (Fig. 6 and Table 1; see also Xiao et al., 1998). Both DP and MK-801 are approximately 3-fold less potent than DM at blocking \( \alpha_3\beta_4 \) receptors. MK-801 has been found previously to block muscle nicotinic receptors (Ramoa et al., 1990; Amador and Dani, 1991) as well as neuronal types of nicotinic receptors (Halliwell et al., 1989; Ramoa et al., 1990; Briggs and McKenna, 1996).

Interestingly, whereas the receptor blockade by mecamylamine was completely reversed by the standard washing conditions used here (four washes over 7 min), DM appeared to require a longer washout period for complete reversal of its effects. Thus, after exposure of cells to DM for 30 min or 24 h, followed by our standard washing procedure, receptor function was still inhibited by 40 to 60% compared with controls. However, the receptor function returned to control values within 2 h after removing the drug, even after a 5-day exposure to DM. One possible explanation for these results is that DM may bind to a site deep within the receptor channel and, even though the affinity of DM for its binding site may be lower than that of mecamylamine, it may not exit the channel as easily.

As a consequence of the relatively slow recovery from exposure to DM, it is uncertain whether the diminished response to nicotine seen in the whole cell patch-clamp studies 8 min after exposure of cells to DM plus nicotine (Fig. 2c) was due to residual blockade by DM or desensitization of the receptor after exposure to nicotine. In other studies, we found that the \( \alpha_3\beta_4 \) receptors in these cells do, in fact, desensitize during acute exposure to nicotine and that the half-time for recovery of their function after a 5-min exposure to nicotine is approximately 8 min (E. L. Meyer, Y. Xiao, and K. J. Kellar, in preparation).

Although DM and DP are structurally similar to many opioid drugs, they do not share certain key pharmacological properties of the opioids. Thus, DM and DP are not by themselves potent analgesics, and they are not usually associated with addictive behavior. DM, however, does appear to potentiate analgesia produced in rodents by both opiate drugs (Elliott et al., 1994; Mao et al., 1996) and nonsteroidal anti-inflammatory drugs (Price et al., 1996). In addition, DM, like codeine and other opiates, is a good cough suppressant; but interestingly, although the opiate receptor antagonist naloxone blocks the antitussive effect of codeine, it does not block...
that of DM (Reisine and Pasternak, 1996). Thus, cough suppression probably involves more than one kind of mechanism, including one that might be mediated by a nicotinic receptor. Interestingly, nicotinic receptors have recently been found in human and rodent bronchial epithelial tissue (Zia et al., 1997; Maus et al., 1998), a location that could make them a ready target of DM in its cough suppression action.

The ability of DM to enter the central nervous system and to block neuronal nicotinic receptors could contribute to its actions to both potentiate analgesic activity and suppress cough, either centrally or peripherally. In addition, recent studies from Rose and colleagues (Rose et al., 1994, 1998) have suggested that nicotinic receptor antagonists, such as mecamylamine, when combined with the nicotine transdermal patch, may be useful as adjuncts treating nicotine addiction. Because DM produces a sustained but reversible noncompetitive block of neuronal nicotinic receptors, it too could complement the activity of other smoking cessation approaches, such as nicotine replacement therapy and bupropion. In this regard, it should be noted that there has been a long period of experience with DM as a cough suppressant (>40 years) and that it has a high safety index (Bem and Peck, 1992). Furthermore, because the complex behavioral effects of nicotine may involve downstream transsynaptic actions of excitatory amino acids at NMDA receptors (Shoaib and Stolerman, 1992; Shoaib et al., 1994), the concurrent blockade of both nicotinic and NMDA receptors by DM and DP may be additive in producing beneficial pharmacological effects. In this regard, it should be pointed out that the combined plasma concentrations of DM and DP vary widely, but can reach 1 μM after a single 30-mg oral dose (Capon et al., 1996); however, the drug is often taken 6 or more times per day and often at higher doses. Furthermore, the brain level of a lipophilic drug like DM may be significantly higher than its plasma level and, in any case, the pharmacological effects of a noncompetitive blocker may be greater than would be predicted by its blood level because it is not competing with an endogenous agonist. In addition, as shown here, the effects of DM appear to reverse relatively slowly.

In conclusion, DM and DP are noncompetitive blockers of neuronal nicotinic receptors composed of α3β4 subunits. The blockade produced by DM is reversible but is sustained beyond the time typically needed to remove drugs from the incubation media, suggesting that DM binds to a site deep inside the channel or otherwise finds a depot away from the surrounding media, perhaps within the cell. The blockade of neuronal nicotinic receptors by DM and its metabolite DP may contribute to their clinical utility as cough suppressants, as well as to their potential use as analgesic boosters and for smoking cessation therapy.

References