Opioid Agonists Modulate Release of Neurotransmitters in Bovine Trachealis Muscle

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Background: Stimulation of opioid receptors in the airways can modulate cholinergic neurotransmission and thereby reduce bronchoconstriction. This protecting effect of opioids against bronchoconstriction may be of clinical interest. Inhalation of opioids as a method of analgesia is likely to result in an opioid concentration at airway receptors sufficient to protect against bronchoconstriction; the concentration may be insufficient when opioids are administered by conventional techniques. In addition, new selective opioids may be developed that could more selectively protect the airways against bronchoconstriction.

Methods: The effect of three selective opioid agonists on the contractile response to electric field stimulation (EFS) was studied in isolated muscle strips from four regions of the bovine trachea (upper, or laryngeal; upper middle; lower middle; lower, or carinal).

Results: The selective κ agonist trans-3,4-dichloro-N-methyl-N-(2-1-pyridilidinyl) cyclohexyl benzene acetamide (U 50488 H) and the selective µ-opioid agonist d-Ala²-N-MePhe³-Gly-ol⁴-enkephalin (DAMGO) reduced significantly (P < 0.001 and P < 0.001, respectively) the contractile response to EFS. The attenuation of the contractile response by U 50488 H was concentration-dependent (P < 0.0001) and tended to be larger at low stimulating frequencies (P < 0.055). The attenuation of the contractile response by DAMGO was frequency-dependent (P < 0.01). The selective δ-opioid agonist α-penicillamine³-α-penicillamine³-enkephalin had no significant effect on the contractile response to EFS (P = 0.71). There were no significant differences among the four regions of the trachea in their responses to the selective opioid agonists U 50488 H (P = 0.50) and DAMGO (P = 0.44). Neither U 50488 H nor DAMGO altered the contractile response to acetylcholine (P > 0.11, P > 0.21, respectively), suggesting that the opioid agonists have a prejunctional effect. The attenuation of the contractile response to EFS by U 50488 H was partially but significantly antagonized by 10⁻³ m naloxone (P < 0.01) and by 10⁻² and 10⁻¹ m of the selective κ-opioid antagonist 2,2’-[1,1’-biphenyl] 4,4-diyil-bis [2-hydroxy-4,4-dimethyl-morpholinium] (P < 0.05). Naloxone (10⁻³ m) abolished the inhibitory effect of DAMGO, suggesting that opioid receptors are involved in the attenuation of the contractile response to EFS afforded by DAMGO and U 50488 H.

Conclusions: We conclude that prejunctional κ- and µ-opioid receptors attenuate the contractile response of isolated bovine trachealis muscle to EFS by inhibiting cholinergic neurotransmission. This effect is uniform throughout the trachealis muscle. δ-Opioid receptors are apparently not present in the bovine trachealis muscle. Caution must be used in extrapolating these results to the intact human. In this study little or no inhibitory effect of the opioids was observed at concentrations expected at airway receptor sites when administered by conventional techniques. However, the effect may be large enough to protect against bronchoconstriction when nebulized opioids are administered by inhalation. (Key words: Agonists, opioid: DAMGO; DPDPF; U 50488 H. Antagonists, opioid: naloxone; nor-BNI. Receptors, opioid: δ, κ, µ. Trachea: bovine.)

IN 1990 Hirshman and Bergman concluded in an authoritative review that the effects of opioids on airway caliber "are small and inconsistent." It has now become apparent that opioids can significantly and consistently inhibit contraction of airways. Also, inhalation of opioids as a method of analgesia may result in higher concentrations at airway receptors than achieved with conventional administration, making protection against bronchoconstriction by inhalation of opioids possible.

Electric field stimulation (EFS) activates nerves in the airway tissue sample to release neurotransmitters in combinations dependent on species and site from which the tissue sample was collected. In isolated canine trachealis muscle the δ-opioid agonists [Leu⁵]-enkephalin and [Met⁵]-enkephalin and the µ-opioid agonists morphine and fenetyl inhibit the contractile response to EFS. The effect of κ-opioid agonists has to our knowledge not been studied in canine trachealis.

The isolated guinea pig trachealis muscle sampled from the lower trachea responds to EFS with an im-
mediate cholinergic and a longer lasting excitatory nonadrenergic–noncholinergic (eNANC) contractile response. Both these responses are attenuated by the selective μ-opioid agonist D-Ala²-N-MePhe⁴-Gly-ol⁵-enkephalin (DAMGO). However, in the upper trachea from guinea pigs, there is no eNANC response. DAMGO has no effect on the EFS-evoked cholinergic contraction. It has therefore been postulated that DAMGO may exert its inhibitory effect partly by inhibition of the facilitatory action on the cholinergic neurotransmission exerted by the eNANC nervous system. Again the effect of κ agonists has to our knowledge not been studied in guinea pig trachealis.

Neither the selective δ-opioid agonist β-phenilethylamine⁶-δ-enkephalin (DPDPE) nor the selective κ agonist trans-3,4-dichloro-N-methyl-N-(2-iminopropyl) cyclohexyl benzene acetamide (U-50488 H) interferes in the isolated human tracheal muscle with release of acetylcholine (ACH) in response to EFS, suggesting that δ and κ receptors may not be present in the human trachealis.

One concludes that knowledge of the effects of opioid agonists on the contractile response to EFS in isolated trachealis is incomplete. Effects of κ-opioid agonists alone and comparative effects from stimulation of all three opioid receptor types (μ, δ, and κ) in one species have not been reported.

We therefore studied systematically the effects of selective opioid agonists for μ, δ, and κ receptors in the isolated bovine tracheal muscle. The bovine trachealis muscle was chosen because its nervous system is similar to that of human airways. In both species the excitatory innervation is predominantly cholinergic; and the inhibitory innervation is predominantly inhibitory nonadrenergic–noncholinergic. Equally important, the bovine trachealis has few or no eNANC nerves, thus permitting by exclusion the importance of the facilitatory role of the eNANC nerves in the attenuation of cholinergic neurotransmission.

Materials and Methods
Bovine tracheas were obtained from an abattoir. They were removed immediately after death from adult animals and immersed in chilled (4°C) physiologic salt solution (PSS) of the following millimolar composition: MgSO₄, 0.8; KH₂PO₄, 1.2; KCl, 3.4; CaCl₂, 2.4; NaCl 110.5; NaHCO₃, 25.7; and dextrose 5.6. Tracheas were transported to the laboratory within 1 h of death. The mucosa (epithelium, basement membrane, and mucosa) was removed and the tracheas (approximately 40 cm long) were divided into four regions 10 cm in length. Two rectangular strips (2 × 15 mm) of tracheal muscle were dissected from each of the upper (arytenoid), upper middle, lower middle, and lower (carinal) regions of the tracheas. The eight muscle strips were mounted in 25-mL water-jacketed glass tissue baths containing PSS at 37°C. The PSS was aerated with a gas mixture containing 95% O₂ and 5% CO₂. One end of the strip was connected to a stationary hook and the other end to a force transducer (FT 03 D, Grass Medical Instruments, Quincy, MA) mounted on a micro manipulator. Isometric forces were recorded continuously (TA 4000, Gould, Valley View, OH).

The strips were contracted every 5 min for 30 s by EFS (25 Hz, 25 V, 0.5 ms). EFS was provided by a direct current amplifier (Department of Engineering, Mayo Clinic, Rochester, MN) triggered by a stimulator (S 44, Grass Medical Instruments) through two vertically mounted parallel platinum electrodes (1 × 4 cm). The strips were stretched progressively after each stimulation by means of the micromanipulator until constant and maximal contractile forces were obtained (optimal length). The strips were kept at this optimal length throughout the study. At the end of the study all muscle strips were blotted dry and weighed, after ties and excessive tissue had been removed.

**The Selective κ-Opioid Agonist U-50488 H Electric Field Stimulation.** Two strips from each of the four regions of the tracheas from six animals were incubated with 10⁻⁶ m propranolol and 10⁻⁵ m indomethacin. Indomethacin was used to antagonize the effect of endogenously produced prostaglandins. After 30 min of incubation all strips were contracted for 30 s with EFS (25 V, 0.5 ms), varying the stimulating frequency in random order between 0.1 and 32 Hz. After completion of this frequency–response study all strips were washed with PSS and reincubated for 50 min with 10⁻⁶ m propranolol and 10⁻⁵ m indomethacin. One strip from each of the four regions was additionally incubated for 10 min with 10⁻³ m U-50488 H; the other four strips served as time controls. All strips were contracted once more by EFS at frequencies ranging from 0.1 to 32 Hz delivered in random order. After completing this second frequency–response curves, all eight strips were incubated for 30 min with 10⁻³ m naloxone and a third complete set of frequency–response curves.
complete set of frequency–response curves was obtained.

**U-50488 H Concentration–Response Curve.** Two strips from the middle lower section of the tracheas from each of another seven animals were used to determine the concentration-response to U-50488 H. Before incubation with U-50488 H frequency–response curves (32, 8, 2, 0.5 Hz) were obtained as base line control. The strips were then washed. One strip from each animal was incubated for 10 min with cumulatively increasing concentrations of U-50488 H (10⁻⁸–10⁻⁴ M) in log increments. After the addition of each aliquot of U-50488 H frequency–response curves were obtained. Simultaneously, time-control frequency–response curves were obtained from the strip from each animal that had not been exposed to U-50488 H.

**Nor-BNI Concentration–Response Curve.** Two strips from the middle lower section of the tracheas from another four animals were used to determine the effect of the highly selective κ antagonist 2,2'-[1,1'-bi-phenyl] 4,4'-dihydro-4,4'-dimethyl-morpholinum (nor-BNI). Before exposure to nor-BNI frequency–response curves (32, 8, 2, 0.5 Hz) were again obtained from all strips. After washing, one strip from each animal served as a control for the effect of time, while the other strip from each animal was incubated with 10⁻⁵ M U-50488 H. A second set of frequency–response curves was then obtained. Thereafter the strips were incubated for 30 min with cumulatively increasing concentrations of nor-BNI (10⁻⁸–10⁻⁵ M) in log increments. Frequency–response curves were simultaneously obtained at each concentration of nor-BNI from the nor-BNI–exposed strips and the time-control strips.

**Acetylcholine.** Two strips from each of the four regions of the tracheas from another seven animals were incubated for 50 min with 10⁻⁷ M tetrodotoxin (TTX). TTX was added to the PSS to interrupt conduction in postganglionic nerves, so that stimulation of muscarinic receptors located in the intramural ganglia cannot participate in the contractile response. Cumulative concentration–response curves to ACh were obtained from all eight strips by increasing the concentration of ACh from 10⁻⁹ to 10⁻⁴ M in half-log increments. Thereafter all strips were washed until the forces had returned to the baseline resting forces. All strips were then re-incubated with TTX (10⁻⁶ M) and one strip from each of the four regions was in addition incubated for 10 min with 10⁻⁵ M U-50488 H. The other strips from the four regions served as time controls. Ten minutes later the concentration–response curve to ACh was repeated in all eight strips.

**The Selective µ-Opioid Agonist DAMGO Electric Field Stimulation.** To determine the effects of DAMGO two strips from each of the four regions of the tracheas from another six animals were used. The protocol was identical to that used in the strips in which the effect of U-50488 H was determined (see above).

**Acetylcholine.** Two strips from each of the four tracheal regions from another six animals were used. The protocol for studying the effect of DAMGO was identical to that described above for determining the effect of U-50488 H.

**The Selective δ-Opioid Agonist DPDPE Electric Field Stimulation.** Two strips from each of the four regions from another four animals were incubated for 30 min with 10⁻⁶ M propranolol and 10⁻⁵ M indomethacin. Baseline frequency–response curves to EFS (0.1–32 Hz) were first obtained. The eight strips were then washed and re-incubated with 10⁻⁷ M propranolol and 10⁻⁵ M indomethacin and one strip from each of the four regions was incubated additionally for 10 min with 10⁻⁵ M DPDPE before a second complete set of frequency–response curves was obtained. The other strips from each of the four regions served as time controls.

**Data Analysis**

All data are expressed as means ± SE. The isometric forces after incubation with U-50488 H, DAMGO, or DPDPE were corrected for the effect of time by the following equation:

\[ T_2 = D_1(D_2/D_1 + [1 - C_2/C_1]) \]  

where \( T_2 \) = the time-corrected response; \( D_1 \) and \( D_2 \) = the contractile responses before and after incubation with U-50488 H; and DAMGO, or DPDPE and \( C_1 \) and \( C_2 \) = the appropriate contractile responses of the control muscles.

The isometric forces after U-50488 H plus naloxone, U-50488 H plus nor-BNI, or DAMGO plus naloxone were corrected for the effect of time by the following equation:

\[ T_3 = D_1(D_3/D_1 + [1 - C_3/C_1]) \]  

where \( T_3 \) = the time-corrected response; \( D_1 \) = the response after incubation with U-50488 H plus naloxone, U-50488 H plus nor-BNI, or DAMGO plus naloxone;
and $C_0$ is the appropriate contractile response of the control muscle.

**Statistical Analysis.** The effects of stimulating frequency, of concentrations of $\text{ACH}$, of U-50488 H, of nor-BNI, of DAMGO, and of DPDPE and the effect of localization within the trachealis muscle were tested for statistical significance by repeated measures analysis of variance and one-sample $t$ tests. For the comparisons of the effects between equimolar concentrations of N-50488 H and DAMGO, and between DAMGO and DPDPE, analyses of variance and two-sample $t$ tests were used. A $P$ value of $<0.05$ was considered to be significant.

**Drugs**

U-50488 H was supplied by Upjohn Company (Kalamazoo, MI). DAMGO and DPDPE were purchased from Bachem, Feinchemikalien AG (Bubendorf, Switzerland). $\text{ACH}$ chloride, indomethacin, $\alpha_1$-propranolol hydrochloride, TTX, nor-BNI, and naloxxone hydrochloride were purchased from Sigma Chemical (Milano, Italy).

U-50488 H was stored at $-4^\circ$C and DAMGO and DPDPE were both stored at $-20^\circ$C. Fresh solutions of all opioid agonists were prepared daily and the tissue baths were wrapped with aluminum foil to avoid degradation by light exposure. All drugs were dissolved in distilled water and added in 100-$\mu$l aliquots to the PSS.

**Results**

Two hundred fifty-four muscle strips from 40 animals were used. The mean weights, resting forces, and maximal forces are given in the table. Incubation with propranolol ($10^{-6}$ m), indomethacin ($10^{-5}$ m), U-50488 H ($10^{-7} - 10^{-3}$ m), DAMGO ($10^{-5}$ m), DPDPE ($10^{-5}$ m) had no discernible effect on the resting force. Spontaneous contractions occurred in 27 strips from 12 animals; the results from four strips could not be used. EFS caused a frequency-dependent contractile response, which was abolished by atropine ($10^{-6}$ m), indicating the response to be solely cholinergic.

**The Selective $\kappa$-Opioid Agonist U-50488 H**

**Electric Field Stimulation.** U-50488 H ($10^{-5}$ m) significantly reduced ($P<0.001$) the contractile response to EFS at all stimulating frequencies (0.1 - 32 Hz) (fig. 1). There appeared to be a trend for a larger attenuation at low stimulating frequencies ($P=0.05$). There were no significant differences ($P=0.50$) among the four regions of the trachea (upper, upper middle, lower middle, and lower) in attenuation of the contractile forces in response to EFS (fig. 1).

Attenuation of the contractile response to EFS by U-50488 H was concentration-dependent ($P<0.0001$) (fig. 2). A significant interaction existed between the concentration of U-50488 H and stimulating frequency ($P<0.01$), indicating that the effect of concentration of U-50488 H was dependent on the stimulating frequency (fig. 2).

Naloxone ($10^{-5}$ m) significantly antagonized ($P<0.01$) the effect of U-50488 H ($10^{-5}$ m) (fig. 3), but antagonism was incomplete. The highly selective $\kappa$-opioid agonist nor-BNI, at concentrations of $10^{-5}$ m or $10^{-6}$ m significantly antagonized the effect of $10^{-5}$ m U-50488 H in a concentration-dependent ($P<0.05$) and frequency-dependent ($P<0.05$) manner. There was a significant variation in stimulating frequency among the four regions (nor-BNI $P=0.035$). Even at 32 Hz U-50488 H was not able to cause any contraction.

**Acetylcholine.** U-50488 H significantly attenuated ($P<0.11$) the force of $10^{-4}$ m ACh in any of the four regions. There were no differences in the force of $10^{-4}$ m ACh in any of the four regions. The mean $P$ values for all the regions are shown in the figure.

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**Table 1. Characteristics of Bovine Trachealis Muscles**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SE</th>
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</thead>
<tbody>
<tr>
<td>Weight (mg) (n = 40)</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Resting force (g) (n = 40)</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Maximal force (g)† (n = 23)</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Maximal force (g)‡ (n = 13)</td>
<td>30 ± 5</td>
</tr>
</tbody>
</table>

$n =$ number of animals and not strips.

* Maximal force at 32 Hz.

† Maximal force at $10^{-4}$ m ACh.

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and frequency-dependent \((P < 0.0001)\) manner (fig. 4). There was a significant interaction between the stimulating frequency and the concentration of nor-BNI \((P = 0.035)\). Even at \(10^{-5}\) m nor-BNI the effect of U-50488 H was not abolished.

**Acetylcholine.** U-50488 H \((10^{-5}\) m\) did not significantly change \((P > 0.11)\) the contractile response to ACh in any of the four regions of the tracheas (fig. 5, average data for the four regions).

**The Selective \(\mu\)-Opioid Agonist DAMGO**

**Electric Field Stimulation.** DAMGO \((10^{-6}\) m\) had no significant effect on the contractile response to EFS (data are not shown).

DAMGO \((10^{-5}\) m\) significantly reduced \((P < 0.001)\) the contractile response to EFS (fig. 6) at low but not high frequencies. Attenuation of the contractile response was significantly larger at lower stimulating frequencies \((P < 0.01)\). There were no significant differ-

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Fig. 6. The selective \( \mu \)-opioid agonist DAMGO attenuated significantly the contractile response to electric field stimulation (\( P = 0.001 \)). The attenuation occurred at all frequencies but was significantly larger at lower frequencies (\( P < 0.01 \)). There were no significant differences among the four regions (upper, upper middle, lower middle, and lower) of the isolated trachealis muscle (\( P = 0.44 \)).

Fig. 7. Naloxone (10\(^{-5}\) M) abolished the inhibitory effect of the selective \( \mu \)-opioid agonist DAMGO (10\(^{-5}\) M). There was no significant difference among the four regions of the trachealis. Therefore only the mean data for the four regions are shown. The reversibility of the inhibitory effect of DAMGO by naloxone suggests that an opioid receptor is involved.

Acetylcholine. DAMGO (10\(^{-5}\) M) had no significant effect (\( P > 0.21 \)) on the contractile response to ACh in strips from any of the four regions of the trachea (fig. 8).

**The Selective \( \delta \)-Opioid Agonist DPDPE**

Electric Field Stimulation. DPDPE (10\(^{-5}\) M) had no significant effect on the contractile response to EFS in any of the four regions of the trachealis (\( P = 0.71 \)) (fig. 9).

**Discussion**

The most important findings of this study are: (1) attenuation of the contractile response to EFS by the selective \( \kappa \)-agonist U-50488 H, a significantly smaller attenuation by the selective \( \mu \)-opioid agonist DAMGO and no attenuation by the selective \( \delta \)-opioid agonist DPDPE; (2) the effects were similar in strips from all four regions of the trachea (upper, upper middle, lower middle, and lower); (3) 10\(^{-5}\) M naloxone abolished the attenuation afforded by DAMGO but only partially antagonized the effect of U-50488 H; (4) nor-BNI, a highly selective \( \kappa \)-opioid antagonist reduced the inhibitory effects of U-50488 H in a dose-dependent manner, but this antagonist was not present in the isolated bowel; (5) ACh did not attenuate the contractile response to EFS in the presence of either drug.

**Limitations**

Because we used isolated tracheal preparations, we were unable to determine the role of neurotransmitters in the modulation of contractile responses to EFS. Nevertheless, our data suggest that the effects of DAMGO and DPDPE on the trachealis may be present in the intact animal. Finally, it is also possible that the three opioid agonists have different actions in different preparations.
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Fig. 9. The selective δ-opioid agonist DPDPE (10⁻⁵ M) had no significant effect on the contractile response to electric field stimulation (P = 0.71), suggesting that δ-opioid receptors are not present in the isolated bovine trachealis.

The inhibitory effects of U-50488 H in a concentration-dependent manner, but this antagonism was incomplete; and (5) ACh did not attenuate the contractile response to EFS in the presence of either U-50488 H or DAMGO.

Limitations

Because we used isolated tissue, which has no afferent pathways to the central nervous system and is not exposed to circulating hormones and other humoral substances that may modulate the contractile response in vivo, we cannot extrapolate these results to intact subjects. Furthermore, only trachealis muscle was studied, and a different distribution of opioid receptors may exist in peripheral airways.

In considering our results with the peptides DAMGO and DPDPE it is important to remember that peptidases may be present in the tissue. To minimize inactivation of DAMGO and DPDPE by peptidases, incubation periods of only 10 min were used. Furthermore, only fresh solutions of peptides were used and the tissue baths were wrapped with aluminum foil to minimize degradation by light. In addition, these peptides were stored in sealed vials at −20°C and were not exposed to room air during preparation of the solutions.

Finally, it also is possible that the concentrations of the three opioid agonists at receptor sites were not the same as in the PSS. If concentrations at receptor sites differed from those in PSS, then the quantitative comparison among the three agonists may be imprecise.

Opioid Receptors

Opioid receptors are widely distributed throughout the body. The inhibitory effect of morphine on the contraction of the smooth muscle of the gut is well known. Because opioid receptors are present in the gut, where their stimulation inhibits peristalsis, it is not surprising that opioid receptors are also present in airway smooth muscle, because both structures are derived from the endoderm. Opioid receptors in airway smooth muscle were identified in 1980, but their function there still remains unclear. It has been suggested that during stress, as for instance during exercise, increased concentrations of enkephalins may protect the airway against neurogenic bronchoconstriction and thus reduce the work of breathing.

Three types of opioid receptors, the μ, κ, and δ, with different affinities for various ligands, are known. Stimulation of all three types produces analgesia. μ and δ receptors have been subtyped into μ₁ and μ₂, and the δ₁ and δ₂ subtypes, respectively. The κ receptor possesses three subtypes, the κ₁, κ₂, and κ₃.

Like the muscarinic and adrenergic receptors, opioid receptors consist of seven membrane-spanning sections. Opioid receptors are functionally coupled by G-proteins to adenyl cyclase and reduce intracellular cyclic adenosine monophosphate concentration. Opioid receptors also activate potassium channels, resulting in loss of intracellular potassium and leading to depolarization of the cell membrane. In addition opioid receptors may activate the N-type Ca²⁺-channels.

The Selective Opioid Agonists

Because the contractile response to EFS was abolished by incubation of the muscle strips with atropine, contractions were purely cholinergic. Both U-50488 H (κ agonist) and DAMGO (μ agonist) but not DPDPE (δ agonist) attenuated the contractile response to EFS in trachealis strips in all four regions of the trachea. This suggests that stimulation of κ and μ but not δ receptors inhibits cholinergic neurotransmission in the isolated bovine trachealis. It also demonstrates that this inhibitory effect is not dependent on the eNANC system.

The nonselective opioid antagonist naloxone abolished the inhibitory action of DAMGO and partially antagonized the effect of U-50488 H. This observation confirms that opioid receptors are responsible for the inhibitory effect. It should be noted that κ receptors...
are approximately tenfold less sensitive to naloxone than are μ receptors,15 hence the smaller response of naloxone in strips treated with U-50488 H.

At equimolar concentrations the κ-opioid agonist U-50488 H was significantly more effective in attenuating the contractile response to EFS than the μ-opioid agonist DAMGO (P < 0.0001) and DAMGO was significantly more effective than the δ-opioid agonist DPDPPE (P < 0.0001) (fig. 10). This is in contrast to findings in bronchi of guinea pigs, where μ receptors are primarily responsible for the inhibitory effect on cholinergic neurotransmission.16 One possible explanation for these different findings is that κ receptors are more abundant in the isolated bovine trachealis than are either μ or δ receptors, whereas μ receptors are more abundant in guinea pig bronchi. Radioligand studies could verify this hypothesis. It is known that large differences do exist in the relative abundance of opioid receptors in the central nervous system16 among species.

The inhibitory action of U-50488 H was partially antagonized by incubation of the muscles with the highly selective κ antagonist nor-BNI in a concentration-dependent manner, confirming that κ-opioid receptors are involved in the inhibition of the cholinergic neurotransmission. Because the inhibitory action of U-50488 H was not abolished by nor-BNI, other factors may contribute to this inhibition.

The attenuation of the contractile response to both DAMGO and U-50488 H was frequency-dependent, with opioid agonists exhibiting their inhibitory effects predominantly at lower stimulating frequencies. This may be of functional importance because autonomic neurons discharge at frequencies less than 5 Hz.

The inhibitory effect of opioid agonists on the contractile response to EFS may occur at two levels, directly at the smooth muscle cell17 or through inhibition of neurotransmitter release from nerve endings. To localize the effect of the opioid agonists, muscle strips were incubated with TTX before they were contracted with ACh. In the presence of TTX the contractile response to ACh results only from a direct stimulation of the smooth muscle cell. In the presence of TTX, neither U-50488 H nor DAMGO had any effect on the contractile response to ACh, indicating prejunctional opioid receptors regulate the release of neurotransmitter. This conclusion is consistent with earlier observations, which suggested that opioid receptors are located prejunctionally at sensory nerve endings.15,16 Further studies are necessary to elucidate the underlying mechanisms for the inhibitory effect of the opioid agonists.

Clinical Implications
Caution should be used in extrapolating these data obtained in isolated bovine trachealis to the intact human. First, the abundance of opioid receptors may differ between the trachea and bronchi, and interpretation of the data is complicated by possible interactions between opioid receptors. For instance, κ agonist can antagonize the respiratory depression produced by stimulation of μ receptors.18 Second, concentrations of opioids, when administered by conventional techniques, may be in the range of 10^{-7} M, a concentration at which the effect of DAMGO was negligible and that of U-50488 H was moderate. However, if nebulized opioids are given by inhalation, a significant amount will be deposited in the airways, resulting in concentrations at airway receptors that may have a significant protective effect against bronchoconstriction. It is therefore important for anesthesiologists to recognize that opioid agonists may have effects on airways other than bronchoconstriction induced by release of histamine. The inhibitory effect of opioid agonists on neurogenic bronchoconstriction may explain why in anesthetized dogs morphine produces transient bronchodilation before the onset of bronchoconstriction induced by histamine.19 If selective opioid agonists can also protect the human airway against neurogenic bronchoconstriction, then this may be a property for the design of new opioid agonists.

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bronchoconstriction, then this may become a desirable property for the design of new opioids.

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