Contractile Responses of Canine Tracheal Muscle during Exposure to Fentanyl and Morphine

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The effects of fentanyl on airway smooth muscle are not known. In this report the authors compare the effects of fentanyl and morphine on isolated canine tracheal smooth muscle. The contractile response of tracheal muscle strips to transmural electrical stimulation was attenuated by fentanyl (≥3 x 10⁻⁶ M) and morphine (≥3 x 10⁻⁷ M) in a dose-dependent manner; the attenuation was partially reversed by naloxone. Contractions induced by acetylcholine, histamine, and serotonin were reduced by prior treatment with fentanyl (≥10⁻⁷ M), but not morphine. The fentanyl-induced inhibition was not reversed by naloxone. In tracheal strips already contracted with acetylcholine, histamine, or serotonin, fentanyl produced relaxation, while, in contrast, morphine elicited a further contraction. In K⁺-contracted strips, fentanyl failed to produce relaxation. In tracheal strips sensitized in vitro by rabbit antisera to bovine serum albumin, the addition of antigen (bovine serum albumin) produced a transient contraction, which was suppressed by prior treatment with fentanyl or chlorpheniramine. It may be concluded that fentanyl and morphine interfere with the release of acetylcholine from cholinergic nerves innervating tracheal smooth muscle; the interference appears to be associated mainly with activation of opiate receptors. Fentanyl, but not morphine, appears to possess antimuscarinic, antihistaminergic H₁, and anti-serotonergic actions. This suggests that fentanyl may be preferable for use in asthmatic patients. (Key words: Analgesics, narcotic: fentanyl; morphine. Antagonists, narcotic: naloxone. Lung: trachea.)

Fentanyl together with droperidol is used widely for neuroleptanalgesia. This analgesic interferes with the release of acetylcholine from cholinergic nerves innervating the heart and intestine, and blocks the alpha-adrenoceptors in the rabbit aorta. However, its effects on the autonomic and autacoid systems in other organs such as tracheal smooth muscle have not been clarified.

Morphine is known to release histamine, which constricts tracheal and bronchial smooth muscles; therefore, this analgesic, if used at all in asthmatic patients, should be used with extreme caution. Whether or not such is true in the case of fentanyl has not been determined.

The present study was thus undertaken to elucidate the influences of fentanyl and morphine on the contractile response of tracheal smooth muscles to cholinergic nerve stimulation, acetylcholine, histamine, and serotonin and on the response to an antigen-antibody reaction. The effects of fentanyl and morphine on tracheal muscle tone were also compared.

Methods

Mongrel dogs of both sexes, weighing 7 to 15 kg, were anesthetized with pentobarbital (50 mg/kg, ip) and sacrificed by bleeding from the common carotid arteries. The trachea close to the bifurcation was isolated. Mucous membrane and connective tissue were removed from the membranous portion of tracheal rings. Tracheal muscle strips approximately 15 mm long and 2–3 mm wide were prepared following the method described by Akçası, with minor modifications. The tracheal strips were fixed vertically between hooks in a 20-ml muscle bath containing the nutrient solution. Constituents of the solutions were as follows (mm): Na⁺ 162.1, K⁺ 5.4, Ca²⁺ 2.2, Mg²⁺ 1.0, Cl⁻ 157.0, HCO₃⁻ 14.9, and dextrose 5.6. The upper end of the strips was connected to the lever of a force-
displacement transducer;‡ The resting tension was adjusted to 1.0 g. The bathing media were gassed with a mixture of 95 per cent O₂ and 5 per cent CO₂ and were maintained at 37 ± 0.5° C. The pH of the solution was 7.2 to 7.3. Before the start of experiments, the preparations were allowed to equilibrate for 90 to 120 min, during which time the solution was replaced every 10 to 20 min.

For studies of the responsiveness to transmural electrical stimulation, the tracheal muscle strips were placed between a pair of platinum stimulating electrodes, approximately 2 mm apart. The gaps between the electrodes and the strip (<0.5 mm) were wide enough to allow for undisturbed tracheal contraction and yet sufficiently narrow to permit effective stimulation of intramuscular nerve terminals. The preparations were transmurally stimulated by a train of square pulses. Supramaximal stimuli of 0.3 msec duration were delivered at frequencies of 20, 10, 5, and 2/sec, each train of stimuli consisting of a total of 200 pulses. Transmural stimulations were initially applied repeatedly at a frequency of 20/sec until steady responses at this frequency were attained; the frequency–response relationship was then obtained. Stimulus pulses were provided by an electronic stimulator.§ Tracheal muscle contractions induced by stimulation at 20/sec before the addition of fentanyl or morphine were taken as controls.

Isometric contractions and relaxations were recorded on an ink-writing oscillograph.§ Acetylcholine, histamine and serotonin were added directly to the bathing media in cumulative concentrations. Tensions developed by acetylcholine, 2 × 10⁻⁴ M, histamine, 10⁻³ M, and serotonin 5 × 10⁻⁵ M, in control media were taken as 100 per cent. The dose–response relationship of acetylcholine, histamine, or serotonin and the contractile response to transmural electrical stimulation were obtained after 20-min exposures to blocking agents such as fentanyl, morphine, atropine, chlorpheniramine, an H₁ antagonist, hexamethonium, and tetrodotoxin. Tetrodotoxin was used to determine whether the response to transmural electrical stimulation was mediated by nerve action potentials. The pA₂ values, which represent the negative logarithm to base of 10 of the molar concentration of an antagonist that causes a doubling of the concentration of an agonist to compensate for the action of the antagonist, were estimated from the ratios of median effective concentrations (ED₅₀) of agonists in the presence and absence of antagonist, fentanyl, or atropine. In preparations already contracted with acetylcholine, histamine, serotonin, or K⁺, fentanyl or morphine was added to the bathing medium in cumulative concentrations, and at the end of each series of experiments, papaverine in a concentration of 10⁻⁴ M was added to attain the maximum relaxation. Relaxations induced by the analgesics relative to those induced by papaverine are presented.

Pairs of tracheal muscle strips isolated from the same dog were treated for 60 min with rabbit antiserum to bovine serum albumin (2 mg IgG pro-

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Fig. 2. Responses of a tracheal muscle strip to transmural electrical stimulation in the presence and absence of fentanyl and naloxone. Fentanyl caused inhibition of the response, which was reversed by naloxone.

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Fig. 3. Alteration by morphine of the contractile response of tracheal muscle strips to transmural electrical stimulation and reversal by naloxone of the morphine action. Contractile responses to transmural stimulation at 20/sec in control medium were taken as 100 per cent; mean absolute values in experiments with morphine (left) and morphine plus naloxone (right) were 4.77 ± 0.41 g (n = 12) and 3.89 ± 0.38 g (n = 7), respectively. Morphine (M) in concentrations > 3 × 10^{-7} M significantly attenuated the response to stimulation (P < 0.01); the attenuation was reversed by naloxone (N). Figures in parentheses indicate the numbers of preparations used. Vertical bars represent SEM.

Addition of antigen, compound 48/80, a condensation product of p-methoxymethylphenylethylamine with formaldehyde, was used to liberate histamine from tracheal muscles, and the response to this compound was compared with that to morphine. After the addition of antigen to antiserum-treated preparations or the addition of compound 48/80 or morphine to control preparations, the muscle strips were repeatedly
washed and the effect of antigen, compound 48/80, or morphine was re-examined to determine whether releasable histamine was still contained in the strips.

Results shown in the text and figures are expressed as mean values ± SEM. Statistical analyses were made using the Student t test for paired data. Drugs used were acetylcholine chloride, histamine dihydrochloride, serotonin creatinine sulfate, fentanyl citrate, morphine hydrochloride, naloxone hydrochloride, atropine sulfate, compound 48/80, hexamethonium bromide, chlorpheniramine maleate, and tetrodotoxin.

Results

Effects of Fentanyl and Morphine on the Contractile Response to Transmural Stimulation

Transmural electrical stimulation of tracheal muscle strips produced a transient, frequency-dependent contraction, which was not attenuated by treatment with hexamethonium, 10⁻⁵ M, but was abolished by tetrodotoxin, 10⁻⁷ M, or atropine, 10⁻⁸ M, in six of six preparations.

The addition of fentanyl in concentrations ranging from 10⁻⁹ to 10⁻⁴ M failed to alter the tension of tracheal strips. Treatment for 20 min with fentanyl in concentrations ≥ 3 × 10⁻⁹ M caused a significant decrease in the contractile response to transmural stimulation in a dose-dependent manner (fig. 1, left). Typical recordings of the inhibitory effect of fentanyl are illustrated in figure 2. The fentanyl-induced inhibition was partially reversed by repeated washing of preparations. The inhibition induced by fentanyl, 3 × 10⁻⁸ M, was reversed by naloxone (10⁻⁵ to 10⁻³ M) in a dose-dependent manner (fig. 1, right). Treatment with naloxone alone in concentrations ranging from 10⁻⁵ to 10⁻³ M did not significantly alter the contractile response to transmural stimulation.

Treatment for 20 min with morphine in concentrations of 3 × 10⁻⁷ and 10⁻⁶ M caused dose-related attenuation of the response to transmural electrical stimulation; however, further increase in the concentration to 3 × 10⁻⁶ M did not produce an additional decrease in the response (fig. 3, left). Naloxone (10⁻⁵ and 10⁻⁷ M) partially reversed the inhibitory effect of morphine (fig. 3, right).

Effects of Fentanyl and Morphine on the Contractile Responses to Acetylcholine, Histamine, Serotonin, and K⁺

The addition of acetylcholine in concentrations ranging from 2 × 10⁻⁸ to 2 × 10⁻⁴ M caused a dose-related contraction in strips of the tracheal muscle. Prior treatment for 20 min with fentanyl (10⁻⁷ to 10⁻³ M) shifted the dose–response curve of acetylcholine to the right in a dose-dependent manner (fig. 4, left). However, fentanyl, 3 × 10⁻⁸ M, sufficient to
Figs. 5 (above), 6 (right, above), and 7 (right, below).

Fig. 5. Responses to fentanyl and morphine of tracheal muscle strips contracted with acetylcholine. Relaxations induced by papaverine, $10^{-4}$ M, were taken as 100 per cent; mean absolute values in experiments with fentanyl and morphine were $4.06 \pm 0.91$ g ($n = 6$) and $3.04 \pm 0.59$ g ($n = 7$), respectively. Fentanyl relaxed, but morphine contracted, the tracheal muscle.

Fig. 6. Responses to fentanyl and morphine of tracheal muscle strips contracted with histamine. Relaxations induced by papaverine, $10^{-4}$ M, were taken as 100 per cent; mean absolute values in experiments with fentanyl and morphine were $2.28 \pm 0.19$ g ($n = 8$) and $3.15 \pm 0.56$ g ($n = 9$), respectively. Fentanyl, but not morphine, relaxed the tracheal muscle.

Fig. 7. Responses to fentanyl and morphine of tracheal muscle strips contracted with serotonin. Relaxations induced by papaverine, $10^{-4}$ M, were taken as 100 per cent; mean absolute values in experiments with fentanyl and morphine were $4.98 \pm 0.87$ g ($n = 5$) and $3.44 \pm 0.43$ g ($n = 5$), respectively. Fentanyl relaxed the tracheal muscle with lower concentrations than those of morphine.
significantly attenuate the contractile response to transmural stimulation, failed to alter the dose-response curve of acetylcholine. The inhibitory effect on the response to acetylcholine was neither reversed nor prevented by naloxone, $10^{-6}$ M, but was partially reversed by repeated washing of preparations. Plots of acetylcholine dose ratios against log concentrations of fentanyl and atropine gave straight lines with slopes of $-0.92$ and $-1.10$, respectively. Mean $pA_2$ values of these agents were 7.20 and 9.46, respectively; thus, fentanyl was approximately 1/182 as potent as atropine. In contrast to fentanyl, treatment with morphine in concentrations $\leq 10^{-3}$ M did not significantly influence the contractile response to acetylcholine (fig. 4, right).

Treatment with fentanyl ($10^{-6}$, $3 \times 10^{-6}$, and $10^{-5}$ M) shifted the dose-contraction response curve of serotonin to the right, while morphine $10^{-4}$ M did not affect the response. The $pA_2$ value of fentanyl against serotonin was 6.02. The fentanyl-induced inhibition was not reversed by naloxone $10^{-4}$ M.

The addition of histamine ($2 \times 10^{-6}$ to $10^{-3}$ M) elicited a dose-dependent contraction of tracheal strips. However, the cumulative dose ($2 \times 10^{-4}$ M or more)-response curves were not reproducible. Thus, instead of a dose-response relationship, the response to a single concentration of histamine ($10^{-3}$ M) sufficient to induce 30 to 55 per cent the maximum contraction was obtained; the preparations were washed three times and equilibrated for 40 to 50 min. Responses to histamine $10^{-3}$ M were repeatedly reevaluated. The first, second, and third responses gradually deteriorated, but after a fourth trial of this concentration of histamine, the magnitudes of contractions became identical. Therefore, subsequent experiments with fentanyl or morphine were carried out by taking the fourth responses as controls. Treatment with fentanyl, $10^{-6}$ M, attenuated the contractile response to histamine by 69 ± 8.1 per cent (n = 8), while morphine $\leq 10^{-3}$ M did not significantly influence the response (n = 2). Fentanyl in concentrations $\leq 10^{-6}$ M failed to attenuate the contractile response to K$^+$ in a concentration of 20 mM, sufficient to produce 40 to 50 per cent the maximum contraction induced by K$^+$, 50 mM (n = 8).

## Effects of Fentanyl and Morphine in Strips Contracted with Acetylcholine, Histamine, Serotonin, or K$^+$

In tracheal muscle strips contracted with acetylcholine, $10^{-7}$ M, histamine, $10^{-6}$ M, serotonin, $10^{-7}$ M, or K$^+$, 20 mM, effects of fentanyl ($10^{-7}$ to $10^{-4}$ M) and morphine ($10^{-7}$ to $10^{-3}$ M) were compared. Fentanyl in concentrations $\geq 10^{-4}$ M caused significant relaxation in strips contracted with acetylcholine (fig. 5), histamine (fig. 6), and serotonin (fig. 7). Average $ED_{50}$ values in these strips were $[2.38 \pm 0.46] \times 10^{-7}$ M (n = 6), $[3.35 \pm 0.30] \times 10^{-7}$ M (n = 8), and $[1.64 \pm 0.48] \times 10^{-6}$ M (n = 5), respectively. Fentanyl did not significantly relax the strips contracted with K$^+$.

Morphine produced contraction in preparations contracted with acetylcholine (fig. 5), histamine (fig. 6), or K$^+$, while in serotonin-contracted strips the
analgesic in concentrations $\geq 10^{-4}$ M caused relaxation (fig. 7).

In tracheal strips under resting condition, the addition of morphine, $10^{-4}$ M, and compound 48/80, $10^{-4}$ g/ml, produced transient contraction (1835 $\pm$ 445 mg, n = 3, and 1245 mg, n = 2, respectively). However, the second trial of the same concentration of morphine (n = 3) or compound 48/80 (n = 2) after repeated washing did not elicit contraction, suggesting that releasable histamine was depleted. Treatment with chlorpheniramine, $10^{-6}$ M, a histaminergic H$_1$ antagonist, suppressed or abolished the morphine-induced contraction in three of three strips. Fentanyl, $10^{-8}$ M, also abolished the contraction induced by morphine or compound 48/80.

Effects of Fentanyl on the Response of Sensitized Tracheal Muscle Strips to Antigen

In tracheal muscle strips sensitized in vitro by treatment with rabbit antiserum to bovine serum albumin, the addition of bovine serum albumin in a concentration of $10^{-4}$ g/ml elicited a rapidly developing, transient contraction (fig. 8). In control strips and those treated with fentanyl, $10^{-8}$ M, the contractile responses to first trials of bovine serum albumin were compared. In strips treated with fentanyl, the response to the antigen was markedly attenuated, as shown in figure 8. Contractions induced by bovine serum albumin relative to those induced by histamine, $10^{-3}$ M, were 13.5 $\pm$ 2.8 per cent in seven control strips and 0.7 $\pm$ 0.3 per cent in seven fentanyl-treated strips. Chlorpheniramine, $10^{-8}$ M, also suppressed the response to the antigen.

The second trial of bovine serum albumin failed to contract the tracheal muscles in control preparations. In five strips in which the contractile response to the first trial of the antigen was completely abolished by fentanyl, the addition of compound 48/80 or the antigen (second trial) did not produce contractions, possibly because of a depletion of releasable histamine on the first trial. After repeated washing, the control and fentanyl-treated preparations responded to histamine with contractions to similar extents (fig. 8).

Discussion

Transmural electrical stimulation applied to isolated dog tracheal muscle strips produced a transient contraction that was abolished by atropine and tetradotoxin; therefore, the contraction appears to be due to acetylcholine released from cholinergic nerve terminals innervating tracheal smooth muscle. Fentanyl in concentrations $\geq 3 \times 10^{-6}$ M attenuated the response to transmural neural stimulation in a dose-dependent manner, as did morphine, the attenuation being reversed by naloxone. Contractile responses to exogenously applied acetylcholine were attenuated by fentanyl in concentrations $\geq 10^{-7}$ M. Contractions induced by K$^+$ were unaffected by the analgesic in concentrations $\leq 10^{-6}$ M. These findings suggest that fentanyl in low concentrations ($3 \times 10^{-9}$ to $3 \times 10^{-8}$ M) acts on opiate receptors in cholinergic nerves innervating tracheal muscles, resulting in interference with the release of acetylcholine, as seen in atrial muscles. Fentanyl in higher concentrations ($\geq 10^{-7}$) appears to show an atropine-like, antimuscarinic action, in addition to an inhibition of the release of acetylcholine. Such an antimuscarinic action of fentanyl was not reversed by naloxone, suggesting that the opiate receptor mechanism is not involved.

Contractile responses of tracheal muscle strips to serotonin and histamine were also attenuated by fentanyl. Tracheal muscles contracted with histamine or serotonin relaxed in response to fentanyl, while K$^+$-contracted muscles were not affected by the analgesic. Histaminergic H$_1$ receptors are involved in the histamine-induced bronchoconstriction in man. Thus, histaminergic H$_1$ and serotoninergic blocking actions may be involved in the fentanyl-induced re-

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* Minimum concentrations sufficient to significantly attenuate the response, except †.
† Concentrations causing 50 per cent inhibition of contraction.
lation. In addition, in tracheal muscles sensitized following incubation in vitro with rabbit antiserum to bovine serum albumin, the addition of antigen, bovine serum albumin, caused a transient contraction. This contraction was not reproducible even when the strips were repeatedly washed, and was abolished by fentanyl or chlorpheniramine. The antigen–antibody reaction appears to release histamine, thereby causing contractions of the tracheal muscle; the releasable histamine would be depleted once the reaction is completed. Fentanyl may abolish the contraction by interfering with the action of histamine on receptors of the muscle, but not by reducing the release of histamine, since in preparations in which the contraction induced by antigen was suppressed by fentanyl, the second trial of antigen or the addition of compound 48/80, a histamine releaser, in fentanyl-free medium failed to produce contractions.

Morphine produced a contraction of tracheal muscle strips under resting conditions or partially contracted with tracheoconstrictors, whereas fentanyl relaxed the strips. The morphine-induced contractions were not reproducible, like those induced by the antigen–antibody reaction or by the addition of compound 48/80. Once compound 48/80 was applied, morphine even in the first trial failed to produce tracheal contractions, and, on the other hand, in strips in which contractions were induced by morphine, compound 48/80 failed to contract the tracheal muscle. Morphine-induced contractions were also suppressed by chlorpheniramine. It may thus be concluded that morphine releases histamine from tracheal muscle strips to produce the initial contractions. Such a histamine release has been documented by Feldberg and Paton and Thompson and Walton. In contrast, there was no tracheal muscle contraction in response to fentanyl; moreover, tracheal muscle strips already contracted with acetylcholine or histamine were significantly relaxed by fentanyl in concentrations that were approximately the same as the attained plasma concentration in man (10⁻⁷ M), as roughly estimated from a bolus intravenous injection of a dose of 5 μg/kg body weight (molecular weight, 528; 90 ml/kg is taken as circulating blood volume), although plasma fentanyl rapidly declines. If these in-vitro results are applicable in vivo to human tracheal and bronchial smooth muscles, fentanyl, but not morphine, may be safely used as an analgesic in patients with bronchial asthma.

In Table 1, data obtained from the present and earlier studies with different isolated preparations are summarized. Antagonism of fentanyl against the response to vagal stimulation was greater in tracheal muscles than in atria; however, the potency ratio of fentanyl to morphine did not appreciably differ (50–100:1). So far as concentrations causing 50 per cent depression of contractions evoked by coaxial stimulation of the guinea pig ileum are concerned, fentanyl is 62 times as potent as morphine. Analgesia data indicate that fentanyl is approximately 130 times (on a molar base) as potent as morphine. Only the blockade of responses to vagal or coxal stimulation and the analgesic action were reversed by naloxone. Antagonism of responses of the trachea to acetylcholine, histamine, and serotonin or responses of the aorta to sympathetic nerve stimulation and norepinephrine was seen only with fentanyl, not with morphine within the range of concentrations used. Whether such characteristics of the actions of fentanyl are also seen in man in health and disease remains to be clarified.

References