Fentanyl's Analgesic, Respiratory, and Cardiovascular Actions in Relation to Dose and Plasma Concentration in Unanesthetized Dogs

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Relationships between plasma concentrations of fentanyl and its analgesic, respiratory and cardiovascular effects were determined in unanesthetized dogs. To avoid drug interactions the authors used trained unanesthetized spontaneously breathing dogs. After a control period in the awake state, fentanyl was injected in increasing amounts (2.5, 5, 20, 40, and 100 μg/kg) at 5-min intervals to a cumulative dose of 167.5 μg/kg administered over 20 min and its effects studied on pain responses (heart and blood pressure changes and somatic responses to tail clamping), respiration (respiratory rate, oxygen consumption, blood gas tensions), and circulation (heart rate and blood pressure). Plasma concentration/effect curves were derived by relating the changes in variables from the awake state to the corresponding plasma concentration (range 2–455 ng/ml). Maximum effects occurred at plasma concentrations at and around 30 ng/ml. Oxygen consumption decreased only slightly and remained well above the basal metabolic rate. Cardiac output, heart rate, respiratory rate, and arterial oxygen tension were almost halved during the full action of fentanyl. In dogs, fentanyl's analgesic action cannot be separated from its respiratory and cardiovascular effects. All receptor-mediated effects are maximal at the same plasma concentration, a phenomenon suggesting saturation of the opiate receptors. (Key words: Analgesics: fentanyl. Pharmacodynamics: fentanyl.)

The OPIATE RECEPTOR-ENDOPHIN system appears to be a neuromodulator system in its own right that is linked not only with pain perception but also with other brain functions such as the control of vigilance, respiration, and circulation. Opiates are known to bind to the same receptors. This specific binding increases with the agonist concentration but reaches a maximum, i.e., saturation when all receptors are occupied. Consequently, these receptor-mediated opiate effects also should attain a maximum that, in the absence of toxic side effects, should mark the upper functional limit of the opiate receptor/endorphin system. This presumption was tested in this study by looking at the concentration/effect relationships for fentanyl's analgesic, cardiovascular, and respiratory action. Such information is lacking because of the difficulties in satisfying two requirements: one, the effects should be studied over the entire operating range of the receptor system, i.e., up to saturation (which has not yet been attempted in humans), and, two, there should be no interaction with other drugs, a problem inherent in experiments on anesthetized animals.

With these stipulations in mind, trained, unanesthetized, and spontaneously breathing dogs were studied. We found that fentanyl's analgesic, cardiovascular, and respiratory actions develop in the same range of plasma concentrations.

Materials and Methods

The effects of fentanyl were investigated in 15 experiments on 13 dogs with an average body weight of 22 (±5 SD) kg. The dogs, which were unrestrained and awake during the control period, were trained to lie quietly, in the lateral decubitus position during the experiments.
The common carotid arteries were exteriorized in skin loops to facilitate blood sampling and the recording of arterial blood pressure. Surgery (exteriorization of the carotid arteries and thoracotomy for the implantation of the aortic flow probes) was performed under chloralose anaesthesia (80 mg/kg) at least five weeks before the first study. The cables from the flow probe were led out through a skin tunnel between the scapulae.

The animals received analgesics and antibiotics for the first 3 days after their surgery.

**Measurements**

**Circulatory Effects**

Arterial blood pressure was recorded continuously with a Statham P33D6® transducer via a plastic cannula inserted into the carotid loop, and heart rate was recorded with an EEG-triggered cardiotochometer.

In six experiments on four dogs, stroke volume was derived continuously by an electromagnetic flow meter (Statham SP2200®), which had been implanted around the ascending aorta. The flow meter was calibrated with saline before implantation.

**Respiratory Effects**

Respiratory rate (by a mercury-in-silastic-gauge11 around the thorax) and oxygen consumption (VO₂) were measured continuously. The latter was achieved by passing air at 20 l/min by means of a precision pump through a transparent plastic hood placed over the animal’s head. The oxygen consumption then can be calculated automatically from the air flow and from the O₂ difference (paramagnetic principle) between the inflowing and outflowing air.12 The error of measurement amounted to less than 5%.

Arterial blood gases and the pH were determined intermittently. A total of approximately 100 ml of arterial blood was removed during the course of each experiment for blood gas analysis (1 ml) and for the measurement of fentanyl (5 ml). This was replaced with an equal volume of saline.

**Test for Pain Responses**

Tail clamping with a haemostat was used as a pain stimulus. The clamp was applied for a few seconds. The test was evaluated in terms of the provoked increase in heart rate and arterial pressure.13 The loss of somatic reactions (tail withdrawal, head raising) was included in the protocol.

**Determination of Fentanyl in Plasma**

Plasma was separated by centrifugation and fentanyl (concentrations expressed as base) extracted with a mixture of hexane and ethanol (volume ratio 19:1), the former being purified by passing it twice through a glass column filled with 28/200 mesh silica gel. Extraction and analysis proceeded according to the method described by Gillespie et al.,14 with alfentanil as internal standard. A Perkin-Elmer® gaschromatograph F 22 equipped with a nitrogen/phosphorus detector was used. Separation was achieved with a column (2 m long and 2 mm diameter) packed with 2% OV-17 on a gas chrom Q80/100 mesh. The injector port and detector were maintained at 280°C, the column at 270°C. Flow rates for the carrier gas N₂, O₂, and H₂ were 80, 60, and 8.6 ml/min, respectively. The average recovery of fentanyl was 94 ± 4.5 (SD)% for duplicate estimations of plasma samples with known amounts of fentanyl that were treated in the same manner as the unknown samples.

**Experimental Protocol**

After setting up the recording system, the awake animals remained undisturbed in the lateral decubitus position, with their heads under the plastic hood for a control period of at least 1 h, during which time all variables were recorded continuously. This period enabled the animals to calm down as indicated by the low heart rate, blood pressure, and O₂ consumption levels.

Fentanyl then was injected in increasing amounts (2.5, 5, 20, 40, and 100 µg/kg, respectively) at 5-min intervals so that a total of 167.5 µg/kg was given within 20 min. The time of injection varied between 30 and 45 s.

Blood samples for the determination of gas tensions and fentanyl were taken during the last minute before the injection of fentanyl and at 1, 2, 5, 10, 30, 80, and 140 min after the last injection.

**Data Analysis**

The results are expressed as averages (±SE). Analysis of variance (repeated measure design) with pairwise comparison15 was performed with P < 0.05 considered significant.

**Results**

All the effects of fentanyl occurred in the same dose range. The respiratory (figs. 1A and B) and cardiovascular variables (fig. 1C) changed in a dose-related

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§ Fentanyl Jansen (0.05 mg fentanyl base corresponding to 0.0785 mg fentanyl-dihydrogencitrate per milliliter)
FIG. 1. Respiratory, cardiovascular, and analgesic effects of fentanyl in unanesthetized, spontaneously breathing dogs. Averages (±SE) from nine experiments in nine dogs that were awake during the controls. Increasing doses of fentanyl (2.5, 5, 20, 40, and 100 μg/kg) were injected at 5-min intervals as indicated by the numerals. The data points at the time of injection (marked by arrows) reflect the effects of the previous dose. The increase in heart rate and blood pressure to tail clamping with a haemostat was defined as a pain response. Differences to the preinjection controls, X = P < 0.05 and XX = P < 0.01. All effects reached maxima after the fourth dose. The variables returned to their preinjection values between 60–90 min after the last injection. The respiratory, cardiovascular, and analgesic effects of fentanyl occurred in the same dose range.

manner, but all effects attained a maximum or minimum after the fourth injection of fentanyl. They slowly returned to their preinjection values during the next 30 min following the fifth injection.

Fentanyl suppressed the cardiovascular responses to noxious stimulation, as can be seen in figure 1D. The increase in heart rate and blood pressure, which occurred in response to tail clamping, were curtailed with increasing doses of fentanyl and finally were abolished completely after the fourth fentanyl injection, as were the somatic pain reactions (withdrawal of the tail, head raising, opening of the eyes).

Fentanyl induced demonstrable changes in the animals' behavior. The first two injections elicited mild restlessness, with small increases in heart and respiratory rates, but as the doses increased, all animals appeared sedated and finally showed sleep-like behavior usually after the fourth dose.

The fentanyl plasma concentrations for the experiments shown in figure 1 are given in table 1 and figure 2. The plasma concentrations following each injection were both dose and time dependent. The concentration decreased quickly from the level at 1 min (453 ± 2 ng/ml) to 31 (±0.43) ng/ml within 10 min after the last injection but much slower to finally 7 ± 0.07 ng/ml in the following 150 min (figure 2).

In comparing figure 1 and figure 2, two points are noteworthy: first, the absence of any additional change
in variables during the first 5–10 min after the last injection, and, secondly, the gradual return of most of the variables toward their preinjection levels when plasma concentration decayed below approximately 30 ng/ml. It appears, therefore, that plasma concentrations in the order of 30 ng/ml are sufficient to reach the full effects of fentanyl.

The concentration/effect relationships in figure 3 support these conclusions. Here the changes from the control values in the awake state were related to the corresponding plasma concentrations, measured 5 min after the injection of fentanyl. The first effects occurred at plasma concentrations of approximately 5 ng/ml, and all curves have minima or maxima at concentrations in the range of 30 ng/ml.

This is also true for the changes in cardiac output seen in the additional six experiments on four dogs in

<table>
<thead>
<tr>
<th>Dose (µg/kg)</th>
<th>Time after the First Injection (min)</th>
<th>Plasma Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>5</td>
<td>2 (±0.05)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>4 (±0.15)</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>15 (±0.54)</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>51 (±0.82)</td>
</tr>
<tr>
<td>100</td>
<td>21</td>
<td>453 (±2.23)</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>131 (±1.00)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>79 (±1.10)</td>
</tr>
</tbody>
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Averages (±SE) from nine experiments on nine dogs. Plasma samples were taken just before the injections and at 1, 2, and 5 min after the last injection.

figure 4. Following a transient increase at low concentrations, cardiac output decreased in parallel with the bradycardia from 1,760 ml/min (SE ± 160) to 800 ml/min or 45% when the action of fentanyl was attained fully at plasma concentrations of approximately 30 ng/ml. Curiously, in spite of this and also in spite of the marked decrease in arterial P\textsubscript{O\textsubscript{2}}, the O\textsubscript{2} consumption declined only from 5.5 to 4.8 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} (fig. 1). All animals showed periodic breathing (Cheyne–Stoke's) after the fourth injection.

**Discussion**

In unanesthetized, spontaneously breathing dogs, fentanyl exerts its analgesic, respiratory, and cardiovascular effects in the same range of plasma concentrations between 5 to 30 ng/ml (2 to 8 × 10\textsuperscript{-8} molar). Although the brain concentrations are unknown, they are assumed to be in equilibrium with those in the plasma, except for the measurements made at 1 and 2 min after injection: In rabbits, brain and plasma concentrations equilibrate within 5 min after injection and decay in parallel thereafter.\textsuperscript{16} Similarly in dogs, brain concentrations peak 5 min after injection during hypercarbia and within 10 to 15 min during normocarbia,\textsuperscript{17} and the maximum cisternal liquor concentrations occur between 2 and 10 min and decline at the same rate as in plasma.\textsuperscript{9} In humans, there is a close parallel between the EEG effects and plasma concentrations of fentanyl, with half-times for effect and concentration of 4.6 and 8.4 min.\textsuperscript{18} Finally, in the present experiments, the concentration

![Fig. 2. Time course of the fentanyl plasma concentrations following the last injection. Fentanyl (100 µg/kg) was injected as the last of several doses (2.5, 5, 20, and 40 µg/kg), the plasma concentration before injection being indicated by the horizontal line. Averages (±SE) of nine experiments on nine awake dogs. Note both scales are logarithmic. The concentration initially decays steeply from its peak of around 500 to 30 ng/ml within 10 min and then slowly to 7 µg/ml during the next 2 h.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931418/)
Fig. 3. Plasma fentanyl concentration/effect relationships of the respiratory, cardiovascular, and analgesic action in unanesthetized, spontaneously breathing dogs. Averages (±SE) from nine dogs. Changes from the preinjection values in the awake state with the curves drawn by eye. Fentanyl (2.5, 5, 20, 40, and 100 μg/kg) was injected intravenously every 5 min. Open circles represent measurements at 5 min, for which plasma and brain concentrations are presumably in equilibrium. At 1 and 2 min after the last injection, represented by open circles, the equilibrium is less certain. The increase in heart rate and blood pressure to tail clamping with a hemostat was taken as a pain response. X = P < 0.05 and XX = P < 0.01 for differences of effects between one concentration to the next. All curves are in the same concentration range with maxima and minima at fentanyl plasma concentrations at about 50 ng/ml.

Equilibrium between plasma and brain also is indicated by the corresponding plasma concentrations at which the maximum effects occurred during the injection period (5-min values) and after which they diminished in the postinjection period (see figs. 1 and 2). In all, the above observations suggest that the plasma concentrations reported here probably reflect those in the brain.

Bradycardia of similar magnitude to that we observed in association with strong activation of cardiac vagal efferents also was seen in mechanically ventilated dogs. This argues against ventilatory events as causative factors in our study, particularly since moderate hypoxia and mild hypercarbia should elicit tachycardia, not bradycardia. Furthermore, fentanyl induced the same decrease in heart rate after iontophoretic application into the vagal nuclei of anesthetized, ventilated dogs and also during perfusion of the fourth cerebral ventricle in awake dogs. In all of these experiments, the heart rate reached a minimum of about 50 beats/min. This effect could be reversed stereospecifically with naloxone, a finding that suggests opiate receptors as the most likely mediators.

Opiate receptors are known to mediate the respiratory action of opiates, so that it is not an unexpected finding when respiratory and cardiovascular effects are seen to occur in the same range of plasma concentrations.
function. Even at doses far greater than would be necessary to achieve complete analgesia, fentanyl does not jeopardize the adequacy of oxygenation in dogs. It should be stressed, however, that this may hold only under the special experimental conditions of this study in which any interaction between fentanyl, sedatives, and anesthetics was excluded. In anesthetized dogs, \( P_{\text{aCO}_2} \) was reported to increase by 25 mmHg, but an increase of only 15 mmHg occurred in the present experiments.

The slowing of the heart and respiration with the accompanying changes in arterial blood gas tensions resemble the autonomic manifestations of slow wave sleep and thus may be the consequence of fentanyl's hypnotic action. The state of vigilance strongly influences respiratory drug effects, particular opiates, which are much more depressant during sleep than in the awake state. Perhaps it is therefore the combined action of fentanyl with sedatives or anesthetics, or with the spontaneous loss of vigilance, i.e., sleep, that jeopardizes adequate breathing.

It would appear that data comparable to those presented here are not available for humans. However, for complete analgesia, humans apparently require similar plasma fentanyl concentrations of around 30 ng/ml. Opiate receptor-mediated, specific effects occur at plasma concentrations in the \( 10^{-8} \) molar range, i.e., a range sufficient for saturation of the opiate receptor system in rats, whereas nonspecific side effects, as seen, for example, on the myocardium, occur in the \( 10^{-5} \) to the \( 10^{-3} \) molar range.

Fentanyl exerts its receptor-mediated analgesic, respiratory, and cardiovascular actions in an identical range of plasma concentrations so that it seems impossible to separate the desired analgesic effects from the untoward cardiovascular and respiratory side effects. Plasma concentrations in the order of 30 ng/ml are sufficient to reach the full action of fentanyl. This also is corroborated by the observation that enflurane MAC is reduced maximally at these plasma concentrations. It therefore would seem necessary to reconsider the rationale for advocating rather large single doses of such a highly lipophilic and therefore short-acting agent as fentanyl to achieve a long-term effect.

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