Ventilatory Effects of Clonidine Alone and in the Presence of Alfentanil, in Human Volunteers

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Clonidine, an α₂-adrenergic agonist, can potentiate opioid-induced analgesia. In a double-blind placebo-controlled study in human volunteers, we sought to determine whether clonidine also potentiates opioid-induced respiratory depression. Hypercapnic ventilatory responses (minute ventilation, mean inspiratory flow rate, and mouth occlusion pressure) were measured in five healthy male volunteers on two separate occasions (with or without clonidine, ≈ 3.5 μg · kg⁻¹ orally) under the following conditions: baseline, 2 h after clonidine/placebo (alfentanil concentration of 0), and during computer-controlled alfentanil infusions to approximate plasma concentrations of 5, 10, 20, 40, and 80 ng · ml⁻¹. Plasma alfentanil concentrations were measured before and after each rebreathing test, and clonidine concentrations were measured after each rebreathing test. The end-tidal CO₂ (PETCO₂) was measured continuously. Data were analyzed by repeated-measures analysis of variance. The PETCO₂ and measured concentrations of alfentanil were included as covariates, and a compound symmetry error analysis was assumed. Statistical significance was achieved when P < 0.05. For minute ventilation, mean inspiratory flow rate, and mouth occlusion pressure there was a statistically significant relationship to the covariates of PETCO₂ and plasma alfentanil concentration. Clonidine, when compared to placebo, caused a small but significant depression of mean inspiratory flow rate. There was similarly a small, but statistically insignificant, depression of minute ventilation by clonidine. The mouth occlusion pressure was not affected by clonidine treatment. Clonidine treatment did not potentiate alfentanil-induced respiratory depression. Although the combination of an opioid and an α₂-adrenergic agonist may act synergistically for the analgesic response, there is no synergistic effect by this drug combination on respiratory depression.

(Key words: Anesthetics, intravenous alfentanil. Drug interactions. Lungs: ventilation. Measurement techniques: carbon dioxide ventilatory response. Sympathetic nervous system, α₂-adrenergic agonists: clonidine.)

CLONIDINE, an imidazoline compound, is a selective agonist at the α₂ adrenoceptor with an α₂:α₁ selectivity ratio of approximately 200:1. This centrally active α₂ agonist was introduced almost three decades ago for the treatment of hypertension. Although its sedative action is one of its more troubling side effects in hypertensive patients, this feature can be used to advantage in the anesthetic paradigm. Also, the α₂ agonists possess anxiolytic effects that are comparable to those reported with benzodiazepine compounds. Additional benefits resulting from the periorpative use of clonidine include significant decrements in intraoperative requirements for opioids and volatile agents and reduction in hemodynamic fluctuations during induction of anesthesia.

The α₂-adrenergic agonists have long been known to possess analgesic activity. Recently, this has been translated into clinical practice. In experimental pain models, the α₂-adrenergic agonists are as efficacious as are opioids, and there is evidence linking the mechanisms and pathways of opioid- and α₂-adrenergic agonist–induced analgesia. Naloxone, an opioid receptor antagonist, blocks α₂-mediated analgesia. Cross-tolerance to α₂ agonists has been demonstrated in animals made tolerant to the antinociceptive effects of opioids. Also, activation of α₂ adrenoceptors stimulates the release of endogenous opiates. Finally, the molecular mechanisms transducing the cellular responses to α₂-adrenergic agonists and opioids are quite similar.

The clinical utility of the α₂-adrenergic agonist as a periorpative adjunctive agent, in lieu of opioids, may be realized only if its clinical spectrum (efficacy vs. side effects) exceeds that of the opioids. The major side effect of opioids is respiratory depression. Because of similarity in the analgesic mechanisms of the opioids and the α₂ agonists, it is possible that these classes of compounds share other clinical features. Studies of the ventilatory effects of the α₂-adrenergic agonist have been reported recently, and the data are conflicting. In a recent animal study, clonidine was noted to have less respiratory depressant effects than morphine.

Apart from the ventilatory effects of the α₂ agonist alone, a related issue concerns a possible synergistic interaction between α₂-adrenergic agonists and opioids. Therefore, in this study we defined the ventilatory effects of clonidine in human volunteers both as a single agent and in combination with alfentanil. We sought to determine whether clonidine affects the hypercapnic ventilatory response and whether clonidine potentiates respiratory depressant effects of alfentanil.
Materials and Methods

Six normal, consecutively recruited volunteers were studied after giving written informed consent under the guidelines of the Stanford Committee for the Protection of Human Subjects. All subjects were nonsmokers within 10% of ideal body weight and denied taking medications or illicit drugs. At the time of recruitment, subjects underwent an initial urine analysis for toxicologic screen, spirometry (PB 900, Puritan Bennett, Westmont IL), and a trial rebreathing study to familiarize them with the procedure and to assuage anxiety.

Subsequent formal studies commenced at 8 AM. Subjects reported to the laboratory after an overnight fast. An intravenous catheter was sited in a forearm vein for drug and fluid administration at a rate of 1.5 ml·kg⁻¹·h⁻¹. Subjects were encouraged to micturate before the baseline and clonidine hypercapnic ventilatory responses. A catheter was inserted into a radial artery for blood sampling and blood pressure monitoring. Lead II of the ECG and pulse oximetry (SpO₂) were monitored continuously throughout the study. Subjects were fitted with a nose clip and occlusive earplugs and subjected to "white noise."

Rebreathing studies were performed as previously described while the subjects were supine in a quiet, darkened room and had their eyes closed. In brief, subjects breathed into a mouthpiece fitted to a two-way valve with a silent, pneumatically operated occlusion balloon in the inspiratory circuit (Hans Rudolph model 3927). The apparatus consisted of a meteorologic balloon suspended in a rigid acrylic box open to the atmosphere via a pneumotachometer (Hans Rudolph model 3819). The system was calibrated before each session with a 3-l syringe. Manually operated valves opened the inspiratory circuit to either the box (during room air breathing) or the bag (during CO₂ rebreathing). A valve on the expiratory circuit similarly directed expired gas to the room or returned gas to the bag during rebreathing. The bag was filled with 7% CO₂, 43% O₂, and 50% N₂ in a volume 1 l greater than the measured forced vital capacity. Mouth pressure was determined using a differential transducer (Validyne MP45). CO₂ was measured by sampling expired gas with an infrared analyzer, which had been previously calibrated against two reference standards. The dead space of the system was approximately 125 ml, and the inspiratory flow resistance was <1.2 cm H₂O·l⁻¹·s⁻¹.

Subjects breathed room air until they demonstrated a stable PETCO₂ (5–15 min.). After several recordings of resting breathing parameters (12–15 breaths), the valves to the rebreathing circuit were opened. Subjects had previously been instructed to take three deep breaths at this time, initiated by a gentle tap on the shoulder. Data collection began 30 s. later. The inspiratory occlusion balloon was randomly inflated every 6–12 expirations during the rebreathing trial and was rapidly deflated after the initiation of inspiration. The rebreathing trial was terminated after a maximum of 4 min, or when PETCO₂ was > 65 mmHg. Duplicate tests were conducted after an interval of 15 min except during alfentanil infusions (see below). These studies represented the pretreatment hypercapnic ventilatory response measurements.

Subsequently, subjects received a coded oral preparation containing either clonidine ≈ 3.5 µg·kg⁻¹ in starch, cellulose, magnesium stearate, and colloidal silicon dioxide or a matched placebo tablet. Both the subject and the observer were unaware as to the treatment given. Following a 2.5-h equilibration time, resting parameters and hypercapnic ventilatory response were measured in duplicate, and arterial blood was sampled for clonidine using a radioimmunoassay method. The sensitivity of the clonidine assay was 0.1 ng·ml⁻¹ with a coefficient of variation of ≤ 15%. Alfentanil was then infused by a programmable computer-controlled infusion pump (Stanpump™) to achieve five steady-state plasma alfentanil concentrations ranging from 5–80 ng·ml⁻¹ from known pharmacokinetic parameters. The Stanpump™ was run on a Toshiba T3100 microcomputer driving a Bard Chronofuser infusion pump. Each alfentanil concentration was maintained for a period of 10 min, during which time the ventilatory measurements were made. Blood was sampled for plasma alfentanil concentrations before and after each hypercapnic ventilatory response determination. Plasma alfentanil concentrations were determined using a standard radioimmunoassay technique. The sensitivity of this assay was 1.0 ng·ml⁻¹ with a coefficient of variation of ≤ 6%. After at least 7 days, the subject was "crossed over" into the alternative placebo/clonidine treatment and the study repeated.

Flow, mouth pressure, and expired CO₂ were continuously recorded on a strip-chart recorder (Gould 2400) and simultaneously digitized and stored (IBM-PC computer). The inspiratory flow determined by the computer varied by less than 1.0% from volumes delivered by a calibrated 1-l syringe in both the nonrebreathing and the rebreathing modes. The inspiratory flow signal was integrated to derive tidal volume. Values were subsequently corrected for pneumotach alinearity and converted to conditions of body temperature, pressure, saturated (BTPS). Ventilatory parameters and PETCO₂ were determined for each of the three breaths preceding an occluded inspiration. Mouth pressure 100 ms after the beginning of an occluded inspiration was determined electronically. The mouth occlusion pressure timing interval of 100 ms was accurate to within 5 ms.

Data Analysis

Data were analyzed by repeated-measures analysis of variance. Each of the three variables—mean inspiratory
flow rate, minute ventilation, and mouth occlusion pressure—were analyzed separately. The intrasubject factors consisted of the two levels of observation, namely, the presence or absence of clonidine. Observations after administration of the tablet (either placebo or clonidine) and before alfentanil infusion were considered to be alfentanil = 0. The measured levels of CO₂ and alfentanil were included as covariates, and a compound symmetry error structure was assumed. Statistical significance was achieved at $P < 0.05$. Analysis was performed with program 5V from the BMDP Statistical Software package.

The model to which the data was fitted was as follows:

$$E[Y] = \kappa + d_1 + \beta \cdot CO_2 + \gamma \cdot alfentanil + \gamma_1 \cdot alfentanil + \tau \cdot CO_2 \cdot alfentanil + \epsilon$$

where

- $E[Y]$ represents the expected value of dependent variable, namely, mean inspiratory flow, minute volume, or mouth occlusion pressure;
- $\kappa$ is a constant (intercept on the Y axis);
- alfentanil is the plasma concentration of alfentanil in nanograms per milliliter;
- CO₂ is the PetCO₂ measured in mmHg and corrected at BTPS;
- $d_1$ is a categorical variable to represent the two levels of observation, namely, the presence or absence of clonidine;
- $\beta$ is the coefficient of the CO₂ level (the slope of the CO₂ response at alfentanil = 0);
- $\gamma$ is the coefficient of the plasma alfentanil concentration in ng · ml⁻¹, which shows the displacement of the response with increasing concentrations of alfentanil;
- $\gamma_1$ is a categorical variable at two levels, to represent the interactive effect between the presence or absence of clonidine with alfentanil;
- $\tau$ is a coefficient to show the effect of alfentanil concentration on the slope of the CO₂ response curve; and
- $\epsilon$ is the error term.

**Results**

Subjects ranged in age from 25 to 33 yr, and in weight from 64–91 kg. All subjects had spirometric values within normal limits as defined by Morris et al.⁹⁸; vital capacities varied from 99% to 132% of predicted, and the forced expiratory volume in 1 s ranged from 95% to 113% of the predicted value. Urine analysis prior to the study did not reveal concealed consumption of illicit drugs that would interfere with the normal regulation of ventilation.

One subject hyperventilated in all experiments, even when at rest breathing room air. The hyperventilation was observed to begin almost immediately upon placement of the mouthpiece. His resting inspired minute ventilation, while breathing room air, was between 7 and 161 l/min, with respiratory rates between 22 and 36 breaths/min. The tendency to hyperventilate did not diminish with increasing familiarization with the apparatus. We decided, prior to analysis of the results, to delete data derived from this subject, leaving us with a set of five subjects. However, even if the data from this subject are included (as was subsequently examined), there is no change in any of the results.

During the study, no subject developed hypoxia (SpO₂ < 93%) or hypotension (systolic blood pressure < 100 mmHg). Moderate bradycardia, 40–50 beats/min, was observed in all of the subjects after clonidine treatment, but none required intervention. All subjects became noticeably drowsy after clonidine, although all were conscious throughout the respiratory drive assessments, even during the infusions of alfentanil. Two subjects developed nausea at the higher concentrations of alfentanil infusion; these infusions were then stopped, and the data at these particular alfentanil concentrations were excluded from the analysis.

The results of the plasma clonidine concentrations are provided in table 1. The initial clonidine plasma concentrations were slightly lower than those observed during the alfentanil infusion and probably reflect the time to maximal plasma concentration after oral administration.⁹⁹ The measured plasma alfentanil concentrations are shown in table 1. There is a small average increase of 4.2 ± 4.5 ng · ml⁻¹ (mean ± SD) between the initial concentrations ("start"), assayed 5 min after commencement of the alfentanil infusion, and the final concentration ("end"), assayed at the end of the hypercapnic ventilatory response assessment at each alfentanil concentration. The plasma alfentanil concentration at the end of the alfentanil infusion was used as the concentration against which the hypercapnic ventilatory response data was correlated (below), because this concentration is more likely to represent the pertinent alfentanil concentration during the assessment.

**TABLE 1. Plasma Clonidine and Alfentanil Concentration (ng · ml⁻¹) at Different Alfentanil Concentrations**

<table>
<thead>
<tr>
<th>Desired [alfentanil]</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Actual [alfentanil]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beginning ± SD</td>
<td>NA</td>
<td>2.22</td>
<td>19.04</td>
<td>31.28</td>
<td>54.63</td>
<td>92.61</td>
</tr>
<tr>
<td>End ± SD</td>
<td>NA</td>
<td>9.14</td>
<td>17.87</td>
<td>29.69</td>
<td>52.86</td>
<td>90.63</td>
</tr>
<tr>
<td>[Clonidine] ± SD</td>
<td>0.78</td>
<td>1.05</td>
<td>1.00</td>
<td>0.91</td>
<td>0.87</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>NA = not applicable.</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

TABLE 2. Estimates of the Parameters in the Basic Model

<table>
<thead>
<tr>
<th>Estimate [Y]</th>
<th>κ</th>
<th>D,</th>
<th>β</th>
<th>γ</th>
<th>γ,</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean inspiratory flow (l/min)</td>
<td>19.78</td>
<td>±2.40*</td>
<td>0.65*</td>
<td>-0.39*</td>
<td>±0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Minute ventilation (l)</td>
<td>8.48</td>
<td>±0.94</td>
<td>0.52*</td>
<td>-0.28*</td>
<td>±0.010</td>
<td>0.000</td>
</tr>
<tr>
<td>Pₐ,4 (cmH₂O)</td>
<td>1.51</td>
<td>±0.08</td>
<td>0.03*</td>
<td>-0.02*</td>
<td>±0.001</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Results for flow and volume were derived from five subjects, 83 hypercarbic ventilatory responses, a total of 1088 data points.
Results for Pₐ,4 were derived from 5 subjects, 83 hypercarbic ventilatory responses, a total of 366 data points.

There was no difference between the control hypercapnic ventilatory responses or the response under the influence of the placebo tablet at alfentanil = 0 for any of the variables. The results for the estimates of each of the parameters in the regression model are shown in Table 2. There were statistically significant relationships of minute ventilation, inspiratory flow rate, and mouth occlusion pressure to the covariates of PETCO₂ and plasma alfentanil concentration. In the case of mean inspiratory flow, there is a significant difference between responses under the influence of clonidine compared to placebo (D₁ = ±2.4). For each of the other variables (minute ventilation, mouth occlusion pressure), clonidine treatment had no significant effect (D₁ = ±0.94, ±0.08, respectively). The other parameters in the model, namely γ₁ (which would indicate an interaction between clonidine and alfentanil) and r (which would indicate the effect of alfentanil on the slope of the CO₂ response) were not significant for any of the variables.

The slopes of the responses (figs. 1–3) and the mean values (figs. 4–6) for each of the three variables (mean inspiratory flow, minute ventilation, mouth occlusion pressure) at a PETCO₂ of 57 mmHg are depicted figuratively in the presence and absence of clonidine at each alfentanil concentration. The mean values were estimated from the regression lines constructed for each response.

Discussion

The principal finding of the present study is that clonidine does not cause a major degree of respiratory depression in response to increasing PETCO₂. In one of the indices of respiratory drive, mean inspiratory flow rate, there was a small but statistically significant depression due to clonidine, equivalent to that seen with alfentanil alone at a plasma concentration of 12 ng · ml⁻¹. (To put this into further clinical perspective, the plasma concentration of alfentanil required to obtund movement in response to surgical incision during nitrous oxide anesthesia is ≈ 279 ng · ml⁻¹.) This effect of clonidine was not enhanced in the presence of alfentanil, even though clonidine concentrations were somewhat higher at this stage (Table 2).

Data from only five subjects were considered, so there is a possibility that with a larger number of individuals,

![Fig. 1. Effect of alfentanil on inspiratory flow rate response during hypercarbia. The slope (Δ inspiratory flow rate/Δ PETCO₂) was calculated from the hypercarbic ventilatory response curves performed in five subjects at each of six desired alfentanil concentrations in the presence (filled circles) or absence (open circles) of clonidine.](image1)

![Fig. 2. Effect of alfentanil on ventilatory response during hypercarbia. The slope (Δ minute volume/Δ PETCO₂) was calculated from the hypercarbic ventilatory response curves performed in five subjects at each of six desired alfentanil concentrations in the presence (filled circles) or absence (open circles) of clonidine.](image2)
clonidine treatment may significantly depress the other indices of respiratory drive (i.e., mouth occlusion pressure and minute ventilation). Indeed, the minute volume was depressed a small but insignificant degree ($P = 0.058$) by clonidine. This degree of depression of minute ventilation is equivalent to the depression seen with alfentanil alone at a concentration of 8 ng·ml$^{-1}$.

Even though clonidine has been used for nearly three decades in the management of hypertension, there are few studies addressing the $\alpha_2$-adrenergic agonists effects on the respiratory system. Hypoxemia follows the intravenous administration of clonidine to sheep. This response is blocked by a peripherally acting $\alpha_2$ adrenoceptor antagonist, suggesting that the response is mediated in part by activation of $\alpha_2$ adrenoceptors on circulating platelets. This hypoxic response appears to be unique to the ungulates. In a recent investigation involving the highly selective $\alpha_2$-adrenergic agonist dexmedetomidine, we found no clinically significant respiratory depression other than that due to its sedative effect.22

Our findings reported here with clonidine are quite similar to those reported recently by Bailey et al.21 inasmuch as there was no significant interaction between clonidine and an opioid. However, in this study we determined a dose-response for alfentanil, and our analysis...
permitted an assessment of the effect of clonidine on this dose-response. In addition, in contrast to the study by Bailey et al.,22 analyzing all of the data by our method does not depend on making any assumptions concerning the error in the estimates of the hypercapnic ventilatory responses. If the slopes were different, this would have been borne out by a statistically significant interactive term between clonidine treatment group and the CO2 coefficient in the model. In our study, there was no significant interactive term between clonidine and the slopes of the CO2 response. Furthermore, our analysis involved fitting a model to measured concentrations of alfentanil, thus allowing for any pharmacokinetic interaction between clonidine and the opioid narcotic. The possibility of such a pharmacokinetic interaction is suggested by our earlier report.7 We should reemphasize that, given the number of subjects (n = 5) and the fact that data were not obtained for two of the subjects at the highest alfentanil concentrations (±80 ng·ml−1), we may still have a type II error in our analytical interpretation.

Our present findings, as well as those of Bailey et al.,22 are at variance with the findings of Penon et al.,21 who reported that epidurally administered clonidine changed the slope of the minute volume versus CO2 curve. However, one cannot deduce from their data whether the minute volume itself is decreased. Since plasma clonidine concentrations were not reported, it is possible that higher clonidine concentrations were obtained than those reported in our study. It is also possible that, even at the same plasma concentration, epidurally administered clonidine exerts a more profound effect on ventilation compared to centrally administered lipophilic opioids.32

Although certain similarities may exist in the mechanism for the antinociceptive action of opioids and α2-adrenergic agonists,14,20 some differences are also apparent.33,34 Even if the two postreceptor–effector mechanisms are similar, the α2-adrenergic receptors may not be present in the respiratory center of the brainstem. There is evidence in support of a synergistic interaction between the α2-adrenergic agonists and opioids in the antinociceptive effects. In a study in cats, Omote et al. demonstrated a synergistic interaction between opioids, acting through the δ opiate receptor, and clonidine on the wide-dynamic-range neuron.35 Osipov et al. delivered a subanalgic combination of intrathecal morphine and clonidine and demonstrated a heightened analgesic response in rodents.17 In a rat model of analgesia, Drasner and Fields demonstrated enhancement of the analgesic response when subanalgic doses of intrathecal clonidine and systemic morphine were administered concurrently.36

Currently, the role of α2-adrenergic agonists is an area of active clinical interest.37 The most likely application may be reductions of the doses of coadministered anesthetic and analgesic agents needed to produce a hemo-
dynamically stable anesthetic.7,38-42 Although there may well be important synergy between α2-adrenergic agonists and opioids, these data suggest that the administration of α2-adrenergic agonists will not potentiate the respiratory depressant properties of coadministered opioid analgesics.

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