Negative Chronotropic Effects of Fentanyl Attenuate Beneficial Effects of Dobutamine on Oxygen Metabolism: Hemodynamic and Pharmacokinetic Interactions

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ABSTRACT

Opioids are well known to cause cardiovascular depression. The aim of the present investigation was to determine whether an interaction of opioid derivatives with catecholamines might be involved in these hemodynamic alterations. Six comatose patients were enrolled into a prospective, nonrandomized pilot trial. All patients first received a continuous i.v. infusion of dobutamine (10 μg · kg⁻¹ · min⁻¹) paralleled by continuous administration of midazolam (0.4 mg · kg⁻¹ · h⁻¹); thereafter, fentanyl was added i.v. (4 μg · kg⁻¹ · h⁻¹). Hemodynamic parameters as well as dobutamine and endogenous catecholamines plasma levels were determined. The mean arterial blood pressure did not change significantly during the whole study period. The continuous administration of dobutamine (steady-state plasma concentrations: 217 ± 118 ng · ml⁻¹) increased the β₁-adrenergic receptor-mediated hemodynamic parameters such as heart rate, stroke volume index, cardiac index, and oxygen delivery index (p < .05). The concomitant administration of fentanyl decreased the heart rate-dependent hemodynamic parameters (p < .05), suggesting that fentanyl antagonizes the chronotropic effects of dobutamine. In parallel, dobutamine plasma levels increased significantly (275 ± 165 ng · ml⁻¹; p < .05). Noteworthy, after administration of fentanyl, oxygen delivery and consumption index returned to baseline values. Radioligand binding experiments on rat cardiac ventricular microsomes ruled out a direct interaction of fentanyl with β₁-adrenergic receptors and, more importantly, a fentanyl-induced inhibition of β₁-adrenergic receptor G protein coupling. Our observations suggest that fentanyl inhibits the frequency-related hemodynamic changes induced by dobutamine. The underlying mechanism is independent of β₁-adrenergic receptors, but is powerful enough to abolish the salutary effect of dobutamine on oxygen delivery and consumption.

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ABBREVIATIONS: ABP, arterial blood pressure; ABPm, mean arterial blood pressure; PAWP, pulmonary arterial wedge pressure; CVP, central venous pressure; CO, cardiac output; CI, cardiac index; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index; SVI, stroke volume index; LVSWI, left ventricular stroke work index; RVSWI, right ventricular stroke work index; DO₂-I, oxygen delivery index; VO₂-I, oxygen consumption index; [¹²⁵I]CYP, (−)-[¹²⁵I]iodocyanopindolol; HRG-complexes, agonist, receptor, and G protein complexes.
Moreover, circulating endogenous opioids are known to be involved in the regulation of blood pressure; in patients suffering from septic shock, β-endorphin concentrations in blood are elevated. Administration of the opioid antagonist naloxone increases blood pressure (Dirksen et al., 1981; Editorial, 1981; Canady et al., 1989). In addition, systemically administered β-endorphin has been shown to produce a naloxone-reversible hypotension (Lemaire et al., 1978).

To achieve adequate sedation and analgesia, high doses of opioid agonists are administered and combinations of benzodiazepines and opioid agonists are often used (Murray and Plevak, 1994). On the other hand, many critically ill patients depend on catecholamines to maintain hemodynamic stability. The aim of the present investigation was to evaluate the pharmacodynamic and pharmacokinetic interactions between the potent opioid derivative fentanyl and the adrenergic agonist dobutamine in critically ill patients. Our study was carried out in sedated patients who received dobutamine and were subsequently treated with fentanyl. We determined the hemodynamic response to this treatment and measured the plasma kinetics of circulating dobutamine and endogenous catecholamines. After an increase in plasma dobutamine levels (after administration of fentanyl) was established, we investigated the interaction between fentanyl and β-adrenergic receptors on rat myocardium. We hypothesized a direct replacement of dobutamine from the receptor or an effect on the β-adrenergic receptor G protein coupling.

**Experimental Procedures**

**Clinical Investigation**

The study protocol was approved by the institutional review board (Ethical Committee of the University Hospital of Vienna); written informed consent was waived.

**Patients.** Six consecutive hemodynamically stable patients, elected from the local Emergency Department, were enrolled in this nonrandomized prospective trial. All patients were unconscious/comatose before initiation of the study (demographic data see Table 1). No baseline sedation was necessary. Exclusion criteria were defined as hemodynamic instability, prior continuous catecholamine application, or opioid medication within the past 24 h. All patients were intubated and subjected to controlled mechanical ventilation. The respiratory settings were not changed during the study period. None of the patients received any medication known to interact with catecholamines or opioids, namely monoamine oxidase inhibitors; α- or β-adrenergic blockers; β-adrenergic agonists other than dobutamine; amphetamine and related compounds; angiotensin-converting enzyme inhibitors; methylxanthines; or antihistaminics.

**Study Protocol.** After insertion of an Edwards Swan-Ganz catheter (Baxter Healthcare Corporation, Edwards Critical-Care Division, Irvine, CA) and an arterial line for continuous invasive blood pressure monitoring, patients first received continuous sedation with i.v. midazolam (Dormicum, Hoffmann-La Roche AG, Basel, Switzerland). This benzodiazepine derivative was administered at a dose of 0.4 mg·kg⁻¹·h⁻¹ with a loading dose of 15 mg as a bolus injection, paralleled by a continuous infusion of dobutamine (Dobutrex, Eli Lilly, Indianapolis, IN) at a dose of 10 μg·kg⁻¹·min⁻¹. Loading doses of midazolam (and thereafter fentanyl) were calculated according to the formula of Ritschel (1986).

Due to the manufacturer’s recommendation and because of feared severe hypotension, the loading dose of fentanyl was reduced to 1.0 mg. Then, the protocol differed for the first two (A, B) versus the final 4 patients (C–F). In patients A and B, i.v. fentanyl (Janssen Pharmaceutica, Beerse, Belgium) was added at a dose of 4 μg·kg⁻¹·h⁻¹ (loading dose 1 mg as a bolus injection) 60 min after initiation of the study and continuously administered at its fixed dose until the end of the observation period. Arterial blood samples were drawn and hemodynamic measurements were evaluated before initiation of the study, and 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, and 240 min thereafter. In patients C to F, the combined treatment with midazolam and dobutamine was extended up to 90 min, and the observation period including the additional administration of fentanyl up to 510 min. Hemodynamic measurements and catecholamine plasma levels were again determined before the initiation of the study, and 30, 60, 90, 150, 210, 270, 330, 420, and 510 min thereafter.

**Hemodynamic Measurements.** The following hemodynamic parameters were evaluated at time points when plasma samples were drawn by use of a HP-CMS-M1166A monitor (Hewlett-Packard-GesmbH, Böblingen, Germany); systolic arterial blood pressure (ABP), diastolic ABP, mean ABP (ABPm), pulmonary ABP, pulmonary arterial wedge pressure (PAWP), and central venous pressure (CVP). Cardiac output (CO) was evaluated by using the thermodilution technique as described previously (Ganz and Swan, 1972). Cardiac index (CI), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), stroke volume index (SVI), left and right ventricular stroke work index (LVSWI, RVSWI), as well as oxygen delivery index (DO₂-I), oxygen consumption index (VO₂-I), and oxygen extraction rate were calculated according to standard formulae (Shoemaker et al., 1974). All indices refer to the patients’ individual body surface area (m⁻²).

**Laboratory Investigations**

**Materials.** Epinephrine, norepinephrine, dopamine, and epinine (deoxyepinephrine) were obtained from Sigma (St. Louis, MO). Water (Rathburn, Walkerton, UK), acetonitrile, and methanol (both from Promochem, Wesel, Germany) were of HPLC grade. All other reagents were supplied by Merck (Darmstadt, Germany) and were of analytical grade. The β-adrenergic antagonist radioligand (−)-[125I]diodocyanopindolol ([125I]CYP; specific activity 2200 Ci/mmol) was obtained from NEN (Boston, MA); GTP-γ-S and HEPES were purchased from Boehringer Mannheim (Mannheim, Germany), and (−)-isoproterenol and d,l-propranolol were obtained from Sigma (St. Louis, MO); all other reagents (analytical grade) were obtained from Merck. Male Rats (200–250 g body weight; Sprague-Dawley) were obtained from the Institute for Animal Breeding (Himberg, Austria).

**Determination of Plasma Catecholamine Levels.** Plasma samples were collected in chilled glass tubes containing glutathione and EGTA (Amersham, Buckinghamshire, UK), and were stored at −70°C until analysis after centrifugation.

Catecholamines (epinephrine, norepinephrine, dopamine, and epine) and dobutamine were determined simultaneously by reversed-phase HPLC as described previously (Alberts et al., 1992) using isoproterenol as an internal standard. Briefly, the analytes were extracted from plasma and derivatized with 1,2-diphenylethylenediamine. Catecholamines were separated on a LiChrospher 100 RP-C Column (Merck), and were detected fluorometrically (excitation wavelength: 350 nm, emission wavelength: 480 nm). The mobile phase consisted of 50 mM sodium acetate buffer, acetonitrile, and methanol (51:41:8). Catecholamines were eluted with a linear acetonitrile gradient within 20 min at a flow rate of 1.0 ml·min⁻¹ (mobile phase at the end of the gradient elution: acetonitrile: methanol = 92:8).

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TABLE 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)/sex</th>
<th>Underlying Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>70/m</td>
<td>St. p. CPR</td>
</tr>
<tr>
<td>B</td>
<td>84/f</td>
<td>Cerebral hemorrhage</td>
</tr>
<tr>
<td>C</td>
<td>51/f</td>
<td>Cerebral hemorrhage</td>
</tr>
<tr>
<td>D</td>
<td>78/m</td>
<td>Apallic syndrome</td>
</tr>
<tr>
<td>E</td>
<td>64/m</td>
<td>Cerebral hemorrhage</td>
</tr>
<tr>
<td>F</td>
<td>76/m</td>
<td>Cerebral hemorrhage</td>
</tr>
</tbody>
</table>

CPR, cardiopulmonary resuscitation.
Preparation of Rat Cardiac Ventricular Membranes. The project was approved by the local Animal Care Committee and was performed at the Department of Pharmacology of the University of Vienna. The preparation of cardiac microsomes from rat ventricles was carried out as described previously (Freissmuth et al., 1986). In brief, ten rats were sacrificed by cervical dislocation; the hearts were rapidly removed and perfused with isotonic saline in a retrograde fashion via Langendorff column until the effluent was clear. Thereafter, the cardiac ventricles were minced in ice-cold buffer (composition in mmol \( \cdot \) liter\(^{-1}\): 20 HEPES-NaOH, pH 7.4, 1 EDTA, 2 MgCl\(_2\), 250 sucrose) and subsequently homogenized by means of an Ultra-Turrax at a tissue-to-volume-ratio of 1:4 at half-maximum speed and 1 \( \times \) 2 s at maximum speed. The resulting homogenate was obtained by differential centrifugation (10 min at 10,000 \( g \) followed by 20 min at 50,000 \( g \)). The resulting pellet was washed three times in sucrose-free buffer, taken up at a protein concentration of 3 mg \( \cdot \) ml\(^{-1}\), snap frozen in liquid nitrogen, and stored at \(-80^\circ\)C. The protein concentration was determined by dye binding with Coomassie Blue using an assay kit provided by BioRad (Richmond, CA).

Radioligand Binding Experiments. In saturation experiments, cardiac ventricular membranes (2.5 \( \mu \)g membrane protein/assay) were incubated in 200 \( \mu \)l reaction buffer (composition in mmol \( \cdot \) liter\(^{-1}\): 50 Tris.HCl, pH 7.4, 1 EDTA, 5 MgCl\(_2\), 1 ascorbate as antioxidant) in the absence and presence of 0.5 \( \mu \)M fentanyl. Increasing concentrations of [\( ^{125}\)I]CYP (from \( \sim 3–300 \) pM, cf. Fig. 3) were added to the reaction mixture. Nonspecific binding was determined in the presence of 3 \( \mu \)M propranolol. Preliminary experiments verified that binding equilibrium was reached within 90 min at \( 30^\circ\)C even at the low concentrations of [\( ^{125}\)I]CYP; binding was stable for at least 2 h (data not shown). After 90 min at \( 30^\circ\)C, the reaction was stopped by filtration over glass fiber filters using a Skatron cell harvester (Skatron, Lier, Norway). The filters were rinsed with 10 ml of ice-cold wash buffer (composition in mmol \( \cdot \) liter\(^{-1}\): 10 Tris \( \cdot \) HCl, pH 7.4, 1 MgCl\(_2\)). The radioactivity trapped on the filters was determined in a gamma counter (Minaxi-γ 5000, Packard) with a counting efficiency of 75%.

Competition experiments were carried out similarly in a final volume of 200 \( \mu \)l containing the reaction buffer described above, 2.5 \( \mu \)g myocardial membrane protein, 60 \( \mu \)M [\( ^{125}\)I]CYP, increasing concentrations of isoproterenol (1 nM–10 \( \mu \)M, cf. Fig. 4). Binding was determined in the absence and presence of 0.5 \( \mu \)M fentanyl and 10 \( \mu \)M GTP\(_{\gamma}\)S. The competition curve can adequately be described by a model assuming two affinity states of the receptor. The high-affinity state reflects the formation of ternary HRG-complexes composed of agonist (H), receptor (R), and G protein (G), in which the agonist is tightly bound (Freissmuth et al., 1989). In contrast, the low-affinity state corresponds to the interaction between agonist and receptor (HR complex). This phenomenon results from formation of the activated, GTP\(_{\gamma}\)S-liganded G protein \( \alpha \) subunits that dissociate from the HRG complex to modulate the activity of effectors (such as adenyl cyclase). Hence, high-affinity HRG complexes are converted to a homogeneous population of low-affinity interaction between agonist and receptor (HR complex). The incubation was done as outlined above for saturation experiments. All experiments were carried out in duplicate.

Statistical Analysis

Statistical calculations were performed using the Statistical Analysis Software package (SAS Institute, Cary, NC). The first two pa-
means. The four patients subjected to the longer study protocol were also analyzed separately; this analysis yielded statistical differences that were similar to those obtained with all six patients (data not shown). The $p$ values in Results therefore represent the whole study population. Values are expressed as the mean ± S.D. A $p$ value less than .05 was considered statistically significant.

The data points obtained in the binding experiments were subjected to nonlinear least-squares curve fitting to the appropriate equations (rectangular hyperbola, Hill equation, displacement from one of two sites) using a Gauss-Newton or Marquart-Levenberg algorithm. Similarly, the increase in dobutamine concentration upon continuous infusion was analyzed by fitting the data to the equation describing a monoexponential rise yielding estimates for the elimination half-life and the steady-state concentration.

**Results**

**Clinical Investigation.** None of the patients had to be withdrawn from the study because of severe hemodynamic instability during the study. Both loading doses, midazolam as well as fentanyl, only led to transient hypotension. We compared hemodynamic parameters before initiation of the study and during i.v. infusions of dobutamine and midazolam. The following changes were observed (see Table 2 and Fig. 1, A-C): heart rate, CI, SVI, and $DO_{2-I}$ increased significantly ($p < .05$), whereas SVRI and PVRI significantly decreased (see Table 2). During continuous i.v. application, dobutamine plasma levels achieved a steady state within 60 min in patients A and B. This steady state could be confirmed in the longer observation period (90 min) in patients C to F.

The administration of i.v. fentanyl in addition to midazolam and dobutamine caused a significant decrease in heart rate, CI, $DO_{2-I}$, and $VO_{2-I}$ ($p < .05$; see Fig. 1, A-D, and Table 2), but no significant change in SVI. The observed increase in SVRI did not reach statistical significance. If the mean values of CI, SVRI, PVRI, and SVI before any drug infusion (baseline values) were compared to those during the combined administration of midazolam, dobutamine, and fentanyl, significant differences were observed. During the latter study period, CI and SVI were higher whereas SVRI and PVRI showed significantly lower values when compared with baseline values ($p < .05$; Table 2). Interestingly, during this study period heart rate, $DO_{2-I}$, and $VO_{2-I}$ returned to baseline values before initiation of therapy (Fig. 1, A, C, and D; Table 2).

When comparing the endogenous catecholamine plasma levels during continuous infusion of dobutamine/midazolam with the combined administration of dobutamine/midazolam/fentanyl, only a transient increase of epinephrine was noted. The levels of the other endogenous catecholamines did not change (Table 3).

**Effect of Fentanyl on Pharmacokinetics of Dobutamine.** In five of the six patients, the continuous infusion of dobutamine resulted in a rapid increase in the plasma concentration (rate constant $\pm$ S.D. = 0.098 ± 0.043 min$^{-1}$). The steady-state plasma levels varied only modestly in these patients (see Fig. 2, A and B; mean steady-state concentration $\pm$ S.D. = 177.8 ± 23.3 ng·ml$^{-1}$). The total body clearance was derived from the constant infusion rate and the steady-state concentration and calculated as 57.3 ± 8.7 ml·min$^{-1}$·kg$^{-1}$. In one patient (patient F, Fig. 2C), however, the increase in dobutamine plasma levels was delayed (rate constant $\pm$ S.E. of the estimate = 0.038 ± 0.03 min$^{-1}$).
Accordingly, the total clearance of this patient was consider-
6
tration consisting of midazolam, dobutamine, and fentanyl (C).

Changes in catecholamine plasma levels before therapy (A), during therapy with midazolam and dobutamine (B), and during the study period consisting of midazolam, dobutamine, and fentanyl (C, D).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before Therapy</th>
<th>Midazolam/ Dobutamine</th>
<th>Midazolam/ Dobutamine/ Fentanyl</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
<td>(A/B)</td>
</tr>
<tr>
<td>Heart rate (beats \cdot min^{-1})</td>
<td>87 ± 23</td>
<td>104 ± 19</td>
<td>86 ± 15</td>
<td>.043</td>
</tr>
<tr>
<td>ABPm (mm Hg)</td>
<td>93 ± 27</td>
<td>72 ± 19</td>
<td>71 ± 13</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mean pulmonary ABP (mm Hg)</td>
<td>24 ± 7</td>
<td>21 ± 5</td>
<td>22 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>PAWP (mm Hg)</td>
<td>13 ± 6</td>
<td>11 ± 6</td>
<td>11 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>7.8 ± 2.4</td>
<td>6.5 ± 0.9</td>
<td>7.2 ± 0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>CI (liter \cdot min^{-1} \cdot m^{-2})</td>
<td>2.8 ± 0.6</td>
<td>4.1 ± 1.3</td>
<td>3.6 ± 1.1</td>
<td>.011</td>
</tr>
<tr>
<td>SVRI (dyne \cdot s \cdot cm^{-5} \cdot m^{-2})</td>
<td>2801 ± 1062</td>
<td>1345 ± 510</td>
<td>1485 ± 444</td>
<td>.035</td>
</tr>
<tr>
<td>PVR (dyne \cdot s \cdot cm^{-5} \cdot m^{-2})</td>
<td>346 ± 120</td>
<td>234 ± 109</td>
<td>254 ± 113</td>
<td>.013</td>
</tr>
<tr>
<td>SVI (cm \cdot contraction \cdot 1 \cdot m^{-2})</td>
<td>33 ± 9</td>
<td>41 ± 14</td>
<td>43 ± 12</td>
<td>.011</td>
</tr>
<tr>
<td>LVSWI (gm \cdot beat^{-1} \cdot m^{-2})</td>
<td>368 ± 10.8</td>
<td>38.9 ± 21.5</td>
<td>37.9 ± 18.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Oxygen extraction O_{2}ER (%)</td>
<td>23.6 ± 5.5</td>
<td>20.6 ± 2.2</td>
<td>20.4 ± 2.8</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. A p value less than .05 was considered statistically significant. n.s., not significant.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before Therapy</th>
<th>Midazolam/ Dobutamine</th>
<th>Midazolam/ Dobutamine/ Fentanyl</th>
<th>Midazolam/ Dobutamine/ Fentanyl (30 min)</th>
<th>Midazolam/ Dobutamine/ Fentanyl</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
<td>(D)</td>
<td>(A/B)</td>
<td>(B/C)</td>
</tr>
<tr>
<td>Dobutamine (ng \cdot ml^{-1})</td>
<td>0</td>
<td>217 ± 118</td>
<td>263 ± 188</td>
<td>275 ± 165</td>
<td>n.s.</td>
<td>.037</td>
</tr>
<tr>
<td>Epinephrine (ng \cdot ml^{-1})</td>
<td>0.2 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.4 ± 0.4</td>
<td>n.s.</td>
<td>.031</td>
</tr>
<tr>
<td>Norepinephrine (ng \cdot ml^{-1})</td>
<td>1.5 ± 1.6</td>
<td>1.6 ± 1.8</td>
<td>0.4 ± 0.6</td>
<td>1.6 ± 2.6</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dopamine (ng \cdot ml^{-1})</td>
<td>3.4 ± 4.2</td>
<td>1.8 ± 2.3</td>
<td>3.2 ± 2.7</td>
<td>2.1 ± 2.7</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Epinephrine (ng \cdot ml^{-1})</td>
<td>3.4 ± 2.2</td>
<td>2.9 ± 2.3</td>
<td>1.4 ± 2.4</td>
<td>2.8 ± 2.6</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. A p value less than .05 was considered statistically significant. (30 min), first point of evaluation after initiation of fentanyl administration.

with a much higher steady-state level (steady-state concentra-
6
tion ± S.E. of the estimate = 53.47 ± 15.6 ng \cdot ml^{-1}).

Accordingly, the total clearance of this patient was consid-
6
erably lower (18.7 ml \cdot min^{-1} \cdot kg^{-1}) than determined for the other five patients.

In all patients, administration of fentanyl led to a slow rise in the plasma concentrations of dobutamine. This effect of fentanyl was first observed in the initial evaluation of two patients (patients A and B; Fig. 2A). However, this increase in dobutamine did not definitively ascertain the achievement of a new steady state over a period of 180 min (Fig. 2A). Hence, in subsequent patients, the administration of fentanyl was started 90 min after the initiation of the dobutamine infusion and the observation period was extended up to additional 420 min; under these conditions, dobutamine-plasma concentra-
6
tions confirmed the new steady-state level (Fig. 2, B and C). This slow rise of dobutamine might be caused by the fact that the loading dose of fentanyl was approximately 70% of the calculated dose necessary to immediately achieve a steady-
state plasma concentration. Although the effect of fentanyl was variable in magnitude, it was observed in all patients, irrespective of whether the infusion of fentanyl had been initiated 60 or 90 min after the administration of dobutamine. Using the Wilcoxon signed rank test for comparing the whole study periods, the increase in dobutamine serum levels was significant (cf. Table 3; steady-state concentration determined before the fentanyl infusion, indicated by the dashed lines in Fig. 2, versus steady-state concentration during fentanyl infusion: 217 ± 118 ng \cdot ml^{-1} versus 275 ± 165 ng \cdot ml^{-1}; p < .05.).

β-Adrenergic Receptor Binding Studies. After the admin-
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istration of fentanyl, the cardiac effects of dobutamine were blunted. In spite of an increase in circulating epineph-
6
rine and dobutamine, the CO was reduced (cf. Tables 2 and 3, and Fig. 1). This indicated that the initial stimulation of cardiac β-adrenergic receptors was counteracted by fentanyl. We therefore used radioligand binding experiments to investi-
6
gate whether fentanyl impairs the coupling of cardiac β-ad-
6
renergic receptors to its cognate G protein G_{s} in rat cardiac ventricular membranes. The β-adrenergic receptors were labeled with the antagonist radioligand [125I]CYP. Saturation experiments were carried out in the absence and presence of fentanyl to confirm that fentanyl does not interact with the ligand binding pocket of the β-adrenergic receptor (Fig. 3); fentanyl decreased the total binding of [125I]CYP (Fig. 3A, ). However, this effect was attributed to a decrease in nonspecific binding (Fig. 3A, ) such that the specific binding calculated for the saturation isotherms in the absence and presence of fentanyl remained unaffected by fentanyl (Fig. 3B, ). Therefore, the binding parameters calculated for the saturation isotherms in the absence and presence of fentanyl were virtually identical (see Fig. 3B; K_{D} = 18.1 ± 2.2 and 17.9 ± 1.8 pM; B_{max} = 281.8 ± 14.5 and 281.8 ± 14.5 fmol \cdot mg^{-1} in the absence and presence of fentanyl respectively).

The effect of fentanyl on the coupling of cardiac β-adre-
6
nergic receptors to G_{s} was determined in competition binding
The present study suggests that fentanyl electively antagonizes, at least in part, various effects that dobutamine exerts on the myocardium by stimulation of cardiac β2-adrenergic receptors (Tuttle and Mils, 1975; Leier and Unverferth, 1983). The actions of dobutamine on the vasculature, i.e., decreases in SVRI and PVRI, attributable to stimulation of β2-receptors (Leier and Unverferth, 1983) and/or blockade of α receptors (Ruffolo et al., 1981), remained unaffected.

Discussion

The administration of opioid derivatives such as fentanyl can result in hypotension. Several mechanisms contribute to this response, including peripheral arterial vasodilation, reduced responsiveness to the baroreceptor reflex, and venous dilatation. However, these effects were not prominent in our patients, because ABPm, SVRI, PAWP, and CVP remained unchanged under fentanyl.

Fentanyl exerts direct effects on the heart. When reviewing the literature, clinical studies and cardiovascular effects of fentanyl reveal different and confusing results: a decrease in heart rate (Liu et al., 1977) was as well observed as an unchanged heart rate (Stanley and Webster, 1978; Bennett and Stanley, 1979; Thomson et al., 1988). Inotropic effects on CO are described as positive or negative (Liu et al., 1977; Bennett and Stanley, 1979). SVRI may decrease (Liu et al., 1977; Bennett and Stanley, 1979) or remain unchanged (Bennett and Stanley, 1979). Accordingly, the exact effects of fentanyl on the heart and vasculature remain obscure.

Benzodiazepines are known to alter hemodynamics: midazolam decreases heart rate, ABPm, PAWP, CI, and SVRI (Massaut et al., 1983). Moreover, negative inotropic effects of fentanyl seem to be enforced by concomitant administration of benzodiazepines (Stanley and Webster, 1978; Heikkila et al., 1984; Thomson et al., 1988). SVRI decreases during combined treatment with benzodiazepines and fentanyl (Stanley and Webster, 1978; Tomich et al., 1983). Interestingly, no cardiovascular depression was noticed when the sympathetic and the parasympathetic tone was eliminated (Flacke et al., 1985).

Our analysis suggests that the change in CI is mainly due to the negative chronotropic effect of fentanyl. The parameters used indirectly to assess the force of ventricular contraction (i.e., SVI, LVSWI, and RVSWI) remained unaffected. In contrast to previous studies, SVRI did not further decrease when fentanyl was administered in addition to midazolam. We therefore attempted to elucidate mechanisms contributing to the observed antagonism between dobutamine and fentanyl. In isolated perfused hearts, morphine and other agonists decreased CO predominantly via depression of the high-affinity ternary HRG complexes (Fig. 4, □) nor affects the ability of GTPγS to destabilize the high-affinity complex (Fig. 4, △), which is required for propagation of the β-adrenergic receptor-dependent transmembrane signaling.
heart rate. This negative chronotropic effect was not relieved by the concomitant application of atropine but was blocked by naloxone (Vargish et al., 1987a,b). The existence of opioid receptors on the myocardium was further substantiated by experiments on cardiac myocytes of rats. Activation of opioid receptors resulted in G$_i$-dependent inhibition of contraction frequency (Ela et al., 1993). As a consequence, the morphine-induced depression of cardiac function was attenuated by naloxone (Vargish et al., 1987b). A direct cross talk between Gi and G$_i$-coupled receptors has been demonstrated to occur in the absence of second messenger synthesis via a membrane-delimited pathway (Ferre ´ et al., 1991) and may involve an intermediate protein that is distinct from G protein subunits and $\beta y$ dimers (Nanoff et al., 1995).

However, our observations clearly show that fentanyl does not affect the ability of $\beta$-adrenergic receptors to couple to their cognate G protein G$_s$. As demonstrated by saturation experiments, a direct replacement on the receptor can be ruled out either.

Noteworthy, the negative chronotropic effect of fentanyl was observed although the levels of circulating dobutamine and epinephrine actually increased. Previous studies have reported clear cut increases in the plasma concentrations of catecholamines after the administration of opioid agonists in human volunteers (Hoee and Duka, 1993) and in animals (May et al., 1988). These changes have been attributed to an action of opioids within the central nervous system. The changes detected in the present study are modest when considering epinephrine and undetectable for norepinephrine. We believe that this difference may be attributable to the fact that we administered fentanyl to sedated patients. It is reasonable to assume that activation of the sympathetic nervous system via a central site of action may be impaired under these conditions. Moreover, all patients have been comatose, which might have further altered hormonal and hemodynamic response by a reduced sympathetic outflow (Flacke et al., 1985).

The steady-state levels of dobutamine ($\sim$180 ng·ml$^{-1}$) and the corresponding total body clearance ($\sim$57 ml·min$^{-1}$·kg$^{-1}$) determined in five patients are in excellent agreement with the parameters reported in literature ($\sim$170 ng·ml$^{-1}$ and $\sim$60 ml·min$^{-1}$·kg$^{-1}$; Kates and Leier, 1978). In one of our patients, the clearance of dobutamine was lower and, accordingly, the steady-state level was higher. This interindividual variability is not unexpected. A previous study indicated that the values determined for the clearance of dobutamine differed up to 5-fold in healthy young volunteers (Berg et al., 1993) and even larger variations have been reported for critically ill pediatric patients (Schwartz et al., 1991). The elimination of dobutamine is rapid; half-lives in the range of 2.4 min were originally calculated from the decay of the plasma concentration after discontinuation of the dobutamine infusion (Kates and Leier, 1978). However, a study on the pharmacokinetics of dobutamine indicated that its elimination is governed by a biexponential function, i.e., the sum of an initial rapid process ($T_{1/2a} = 1.7$ min) and a second phase ($T_{1/2b} = 26$ min); this is consistent with elimination of dobutamine from two compartments (Schwartz et al., 1991). Our blood sampling protocol was not designed to address this issue but to confirm that steady-state values had been reached before the initiation of the fentanyl infusion. Nevertheless, if the half-life is calculated from the rate constant describing the approach to steady state, intermediate values are obtained (mean $\pm$ S.D. = 9.9 $\pm$ 5 min; range 4.7–18 min). It is likely that these half-lives reflect, in fact, an average estimate of the two phases. The infusion of fentanyl increased the plasma concentrations of dobutamine. To the best of our knowledge, drug interactions based on pharmacokinetic interferences have not been documented for fentanyl and dobutamine. The pharmacokinetics of dobutamine are known to be affected by the concomitant administration of dopamine (Schwartz et al., 1991; Eldadah et al., 1991). However, administration of fentanyl did not alter the plasma concentration of endogenous dopamine. Thus, the observed increase might, at least in part, be due to the changes in CO, which can per se account for a reduction in the clearance of dobutamine. However, correlation analysis between the increase of dobutamine plasma concentrations and the decrease in heart rate did not reveal any statistical significance (data not shown). Irrespective of the nature of the underlying process, we stress that the changes in the plasma concentrations of dobutamine induced by fentanyl were modest. Most importantly, these alterations were not accompanied by any increased pharmacodynamic effect of dobutamine.

Summarizing our results, fentanyl in addition to midazolam and dobutamine neither exerted negative isotropic effects on the heart nor decreased SVRI, as expected. We only could observe negative chronotropic effects, which seem to be responsible for wrecking the beneficial effects of dobutamine on oxygen metabolism. Because these hemodynamic alterations were not similar to those usually observed during combined administration of fentanyl and benzodiazepines (Liu et al., 1977; Tomich et al., 1983; Heikkila et al., 1984), we assume that first and foremost fentanyl alone was responsible for the observed sympatholytic effects.

Both dobutamine and potent opioid analgesics such as fentanyl are widely used for hemodynamic support and analgesia as well as sedation in the management of critically ill patients. However, based on our results we conclude that the combined treatment with dobutamine, benzodiazepines, and fentanyl or other opioids should be carefully monitored. This is particularly relevant in patients who depend on the salutary effect of dobutamine on oxygen delivery and consumption (Shoemaker et al., 1974; Shoemaker, 1995), especially those suffering from septic shock, patients with severe cardiac failure (Shoemaker et al., 1991), and patients affected by an acute respiratory distress syndrome (Steltzer et al., 1994). When using dobutamine, the clinician who resorts to the concomitant administration of fentanyl and midazolam should therefore be aware of the functional antagonism exerted by this opioid. A substitution with other agents such as ketamine, which does not decrease CI (Schwartz and Horwitz, 1975) and is discussed to be the anesthetic agent of choice in clinical situations when O$_2$ availability is reduced (Berman et al., 1990), might be preferable. Thus, the leading role of opioids as analgesic agents should be redefined in patients depending on high oxygen delivery and consumption. In contrast, the pharmacokinetic interaction described in the present work, which results in an altered metabolism of dobutamine, is presumably of little clinical relevance in the short-term management of patients.
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