Interaction of physostigmine and alfentanil in a human pain model†

A. P. Wehrfritz, H. Ihmsen, S. Schmidt, C. Müller, J. Filitz, J. Schüttler and W. Koppert

1Department of Anaesthesiology, University Hospital Erlangen, Krankenhausstrasse 12, D-91054, Erlangen, Germany. 2Department of Anesthesiology and Intensive Care, Hannover Medical School, Carl-Neuberg-Strasse 1, D-30625, Hannover, Germany

*Corresponding author. E-mail: koppert.wolfgang@mh-hannover.de

Background. Preclinical and clinical studies suggest an important role for cholinesterase inhibitors in pain therapy. The aim of this study was to examine the analgesic and antihyperalgesic properties of the cholinesterase inhibitor physostigmine and the opioid alfentanil, alone and in combination, in an experimental pain model in humans.

Methods. Twenty healthy volunteers were enrolled in this double-blind and placebo-controlled cross-over study. Transcutaneous electrical stimulation at high current densities induced spontaneous acute pain and stable areas of hyperalgesia for painful mechanical stimuli (pinprick-hyperalgesia). Pain intensities, measured on a numeric rating scale (NRS) from 0 to 10, and the extent of the hyperalgesic areas were assessed before, during, and 145 min after i.v. infusions of physostigmine (30 µg kg⁻¹ in 15 min), alfentanil (20 µg kg⁻¹ in 2 min), the combination of the same doses of both drugs, or saline 0.9%. The type of interaction was determined by fitting an interaction model to the data.

Results. Starting from a baseline value of NRS = 6, the maximum reduction of pain intensity was 50.4 (± 22.3) % after alfentanil, 35.4 (± 20.0) % after physostigmine, and 60.4 (± 17.1) % after the combination. The hyperalgesic areas were reduced by 53.8 (± 33.2) %, 47.0 (± 26.3) %, and 54.8 (± 33.2) %, respectively. The data were best described by a model assuming an infra-additive interaction for analgesic and antihyperalgesic effects.

Conclusions. Physostigmine and alfentanil showed distinct effects on pain and hyperalgesia in a human pain model. The interaction of both drugs was found to be infra-additive.

Br J Anaesth 2010; 104: 359–68

Keywords: pain, experimental; pain, mechanism

Accepted for publication: October 31, 2009

Pain after surgery consists of a number of different components. Among the most important are unpleasant sensory, emotional, and mental experiences associated with neuroendocrine, metabolic, and immune alterations.1 Inadequate pain management can lead to postoperative wound infections, pulmonary and cardiovascular complications,2 chronic pains3 with long-term effects on quality of life and states of confusion, which can be found especially among older patients.4 Thus, sufficient pain therapy is important for patient satisfaction and quality of life and leads to a reduction of postoperative mortality.5 However, pain therapy is often accompanied by several side-effects. Patients receiving opioids in high dosages often show nausea, vomiting, or sedation. In patients undergoing visceral surgery, a delayed return of bowel function and constipation is often reported. As a result, combination of opioids and non-opioids such as non-steroidal anti-inflammatory drugs (NSAIDs), COX-2

†This work was presented in part at the Euroanaesthesia Meeting, 31 May–3 June 2008, Copenhagen, Denmark.

‡These two authors contributed equally to this study.
inhibitors, or paracetamol have been used as part of a multimodal strategy (‘balanced analgesia’) to enhance analgesia while reducing the dose-related side-effects. The non-opioids, however, can also elicit adverse effects especially on the gastrointestinal tract, platelets, and kidney.

Thus, antinociceptive compounds with fewer side-effects would be of value. Among non-opioids, cholinergic drugs such as nicotinic and muscarinic agonists and acetylcholinesterase inhibitors might meet this expectation. From a variety of preclinical studies it is known that the cholinergic system might play an important role in the modulation of pain. Studies on the postsurgical incision model indicate an effective antihyperalgesic impact of nicotinic and muscarinic agonists and acetylcholinesterase inhibitors. Several clinical studies in patients undergoing different types of surgery showed improved relief from pain without side-effects after perioperative administration of acetylcholinesterase inhibitors. In one of these studies, a continuous infusion of 1 mg h \(^{-1}\) physostigmine, an indirect cholinesterase inhibitor, led to a significant reduction of pain and consumption of morphine in patients after gynaecological and visceral surgery.

Therefore, the aim of this study was to characterize the analgesic and antihyperalgesic properties of the cholinesterase inhibitor physostigmine and the opioid alfentanil, alone and in combination, in an experimental model of electrically evoked pain and secondary hyperalgesia. Pharmacodynamic modelling was used to determine the type of interaction of the drugs.

**Methods**

**Subjects**

After approval by the Ethics Committee of the Medical Faculty of the University of Erlangen, 20 healthy volunteers (10 females and 10 males) were enrolled in this randomized, double-blind, and placebo-controlled study in a cross-over design. The median age was 24 years (range 21–33 years), the mean weight was 71 (SD 11) kg, and the mean body-mass index 22.5 (SD 2.0) kg m\(^{-2}\). All subjects were familiarized with the stimulation procedures before participation. Exclusion criteria were a known drug allergy and a history of nicotine, alcohol, or drug abuse. Patients with acute skin disease, lesions, sunburn, or tattoos in the testing area or intake of medication that may have interfered with pain sensations (i.e. analgesics, antihistamines, calcium, or sodium-channel blockers) were excluded. The study was conducted at the Department of Anaesthesiology, University Hospital Erlangen, Germany. Each subject gave informed consent to take part in the study; the experiments were performed in accordance with the Declaration of Helsinki and good clinical practice.

**Experimental pain model**

Intradermal electrical stimulation was used to induce ongoing pain and secondary mechanical hyperalgesia. In this model, mechanical hyperalgesia to pinprick and touch are elicited by axonal electrical stimulation bypassing the sensory endings, showing the central origin of the hyperalgesia. Briefly, two microdialysis fibres equipped with internal stainless steel wires were inserted intracutaneously at a distance of 4 mm apart in the central volar forearm of the subjects. Monophasic, rectangular electrical pulses of 0.5 ms duration were applied with alternating polarity via a constant current stimulator (Digitimer S7, Digitimer, Hertfordshire, UK) at 2 Hz. The current was gradually increased during the first 15 min of stimulus administration, targeting a pain rating of 6 on a 11-point numeric rating scale (NRS; 0=no pain and 10=maximum tolerable pain), and was then kept constant for the remaining time of the experiment. This experimental approach has been proved to provoke stable areas of secondary hyperalgesia to punctate stimuli and touch caused by an activation of primarily mechano-insensitive (‘silent’) C-nociceptors. This class of nociceptors was shown to be electrically activated preferentially at high current densities as used in this model.

**Medication**

Four separate treatment trials were performed, at least 2 weeks apart. To achieve a double-blinded setting, a computer-generated randomization list was drawn up by a statistician and the sequence of the medication was provided according to this list by an investigator not involved in the data acquisition. After a 30 min equilibration period, the subjects received i.v. infusions of 0.9% saline as control (medication 1), 30 µg kg\(^{-1}\) physostigmine (Anticholium®, Dr Koehler Chemie GmbH, Alsbach-Haehnlein, Germany) for 15 min (medication 2), 20 µg kg\(^{-1}\) alfentanil (Rapifen®, Janssen-Cilag GmbH, Neuss, Germany) for 2 min (medication 3), or a combination of both drugs (medication 4) in the same doses and time (Fig. 1). For blinding, two syringes were prepared for each trial. Syringe 1 contained either physostigmine (medications 2 and 4) or saline (medications 1 and 3), and syringe 2 contained either alfentanil (medications 3 and 4) or saline (medications 1 and 2). In each trial, the administration lasted 15 min for syringe 1 and 2 min for syringe 2. The doses of physostigmine and alfentanil were chosen on the basis of clinically used dosages for i.v. administration in adults. Pulse oximetry (Sp\(_O_2\)), ECG, and non-invasive arterial pressure were monitored continuously all 10 min during the study.

**Sensory testing**

An observer who was not involved in the drug administration asked the subject every 5 min to rate the intensity
Interactions of physostigmine and alfentanil

Fig 1 Schematic illustration of the experimental protocol. Four separate treatment trials were performed. The subjects received i.v. infusions of physostigmine, alfentanil, a combination of both, or saline as a control in a randomized order. The drugs were delivered by a continuous infusion lasting 15 min (physostigmine) or 2 min (alfentanil) and starting 30 min (physostigmine) or 35 min (alfentanil) after the onset of the electrical stimulation.

of ongoing pain induced by the electrical stimulation on the NRS (Fig. 1). The area of punctate hyperalgesia was determined with a 256 mN von Frey filament (Stoelting, Chicago, IL, USA), the area of touch-evoked allodynia with a cotton-wool tip gently stroking the skin. The borders of the hyperalgesic areas were determined by moving along four linear paths parallel and vertical to the axis of the forearm from distant starting points towards the stimulation site (step size 0.5 cm), until the volunteer reported increased pain sensations evoked by the von Frey filament (punctate hyperalgesia) or unpleasant sensations by stroking the skin with the cotton wool (allodynia). Areas of pinprick-hyperalgesia and allodynia were repeatedly tested at 20 min intervals (Fig. 1).

Data analysis

For each volunteer and for each treatment (alfentanil, physostigmine, and a combination of both), the relative changes of the pain ratings and the relative changes of the hyperalgesic areas compared with the baseline values were calculated for each measurement. Subsequently, the individual values were corrected for time-related effects (e.g., tolerance or sensitization) by subtracting the corresponding values of the control group. The maximum observed reduction of the pain rating and of the hyperalgesic area was determined to characterize the effect of each treatment.

Pharmacodynamic modelling and characterization of the interaction

The type of interaction between alfentanil and physostigmine was assessed based on the Loewe approach of isoboles. In this approach, the interaction is called additive if the concentrations of drug combinations leading to the same effect lie on a straight line defined by the effective concentrations of the single drugs. The interaction is called supra-additive if the drug combinations lie below, and infra-additive if they lie above this line of additivity. In order to obtain the isoboles for analgesia and antihyperalgesia, we performed a pharmacodynamic modelling. A sigmoid model was fitted to the data:

\[ E = E_{\text{max}} \frac{C_e}{C_e + EC_{50}} \]  

where the effect \( E \) is the percentage change of the pain rating or the hyperalgesic area, respectively, \( E_{\text{max}} \) is the maximum effect, \( EC_{50} \) is the effect site concentration of the drug, \( EC_{50} \) is the half-maximum effect site concentration, and \( y \) is a coefficient describing the steepness of the concentration–effect curve.\(^{19} \) \( E_{\text{max}} \) was set to \(-100\), assuming that both drugs are able to completely suppress pain and hyperalgesia. The effect site concentration \( C_e(t) \) was calculated from the plasma concentration \( C(t) \) by convolution with the effect site disposition function:

\[ C_e(t) = k_{e0} \int_0^t e^{-k_{e0}(t-\tau)} C(\tau) d\tau \]  

where the rate constant \( k_{e0} \) characterizes the equilibration between plasma and effect site concentration.\(^{19} \) As we did not measure plasma concentrations, we used a three-compartment model from Maitre\(^{20} \) and a two-compartment model from Hartvig\(^{21} \) to estimate the plasma concentrations of alfentanil and physostigmine, respectively.

The data for alfentanil, physostigmine, and the combination of both drugs were analysed simultaneously by using an interaction model. We tested two different interaction models. The first model (model 1), which was proposed by Bol and colleagues,\(^{22} \) is able to describe additive, supra-additive, and infra-additive interactions:

\[ C_e = \frac{C_{EA}}{EC_{50A}} + \frac{C_{EP}}{EC_{50P}} + \frac{\rho C_{EA} C_{EP}}{EC_{50A} EC_{50P}} \]  

\[ E = E_{\text{max}} \frac{C_e^y}{1 + C_e^y} \]  

where \( C_{EA} \) and \( C_{EP} \) are the effect site concentrations of alfentanil and physostigmine, and \( EC_{50A} \) and \( EC_{50P} \) are the concentrations for half-maximum effect when either drug is administered alone. For this model, the isobole for half-maximum effect has the form:

\[ \frac{C_{EA}}{EC_{50A}} + \frac{C_{EP}}{EC_{50P}} + \frac{\rho C_{EA} C_{EP}}{EC_{50A} EC_{50P}} = 1 \]  

The isobole can be plotted as a curve with the relative concentrations \( C_{EA}/EC_{50A} \) and \( C_{EP}/EC_{50P} \) on the x- and y-axis, respectively. The single concentrations of either alfentanil or physostigmine, which lead to half-maximum
effect are given by the points (1,0) and (0,1). For this model and following the Loewe definition given above, the interaction is additive if \( e = 0 \), infra-additive if \( e < 0 \), and supra-additive if \( e > 0 \), respectively.

Although this model is a general model for all types of interaction, it has some limitations. First, the parameter \( \gamma \) is assumed to be identical for both drugs which is not necessarily true. Second, as the isobole has the general form \( y = (1 - x)/(1 + e \times x) \), negative values of \( e \) (infra-additive interaction) lead to a non-defined value of \( y \) (singularity) if the denominator becomes zero. We therefore also fitted an alternative model (model 2), which allows different values of \( \gamma \) for the two drugs:

\[
C_E = \left( \frac{C_{EA}}{EC_{50A}} \right)^\gamma + \left( \frac{C_{EP}}{EC_{50P}} \right)^\nu + \varepsilon \left( \frac{C_{EA}}{EC_{50A}} \right)^\gamma \left( \frac{C_{EP}}{EC_{50P}} \right)^\nu
\]

(6)

\[
E = \frac{C_E}{1 + C_E}
\]

(7)

For this model, the isobole for half-maximum effect is defined by the following equation:

\[
\left( \frac{C_{EA}}{EC_{50A}} \right)^\gamma + \left( \frac{C_{EP}}{EC_{50P}} \right)^\nu + \varepsilon \left( \frac{C_{EA}}{EC_{50A}} \right)^\gamma \left( \frac{C_{EP}}{EC_{50P}} \right)^\nu = 1
\]

(8)

With this model, the type of interaction depends not only on \( e \) but also on \( \gamma_A \) and \( \gamma_P \). If \( \gamma_A > 1 \) and \( \gamma_P > 1 \), the isobole is infra-additive even if \( e = 0 \). As an example, for \( \gamma_A = 2, \gamma_P = 2 \) and \( e = 0 \), equation (8) describes a circle. With negative values of \( e \) one yields stronger infra-additivity. Supra-additive interaction is achieved with \( \gamma_A < 1, \gamma_P < 1 \), and \( e > 0 \), and additivity if \( \gamma_A = 1, \gamma_P = 1 \), and \( e = 0 \).

In a first step we analysed the data with model 1. If infra-additivity (\( e < 0 \)) was found with this model, we further tested model 2, starting with the constraint \( e = 0 \) (reduced model 2), and finally we tested whether the addition of the parameter \( e \) improved model 2. A possible effect of gender on \( EC_{50} \) was tested with an additional parameter \( \theta_{SEX} \): \( EC_{50} = EC_{50male} \times (1 + \theta_{SEX} \times SEX_i) \), where \( EC_{50} \) is the \( EC_{50} \) for the \( i \)th individual, \( EC_{50male} \) is the \( EC_{50} \) for males, and \( SEX_i \) defines the gender of the \( i \)th subject (0=male, 1=female).

The pharmacodynamic parameters were estimated by population analysis (NONMEM® Version VI, Level 2.0, GloboMax LLC, Hanover, MD, USA). The NONMEM control file for the final model is given in supplementary appendix 1, and a part of the data file is given in supplementary appendix 2 (please see British Journal of Anaesthesia online). Interindividual variability of the parameters was assumed to be log-normally distributed: \( P_i = P_{TV} \times e^{\eta_i} \), where \( P_i \) is the parameter value in the \( i \)th subject, \( P_{TV} \) is the typical value of the parameter in the population, and \( \eta_i \) is a random variable with a mean of 0 and a variance of \( \omega^2 \). To account for interindividual variability in pharmacokinetics, we also included \( \eta_i \)'s with fixed variances \( \omega^2 \) for clearance and central volume of distribution of alfentanil and physostigmine. For alfentanil, we used the estimates of \( \omega^2 \) reported in the work of Maitre.20 For physostigmine, the variances \( \omega^2 \) for clearance and volume of distribution were approximately estimated from the individual parameter estimates given in the work of Hartvig,21 assuming a log-normal distribution. Residual error was described by an additive error with mean zero and a variance of \( \sigma^2 \), where \( \sigma^2 \) was assumed to be different for alfentanil, physostigmine, and the combination. Initially, the first-order estimation method was used for the analysis. After the best model was selected, the parameters of the model were re-estimated using the first-order conditional estimation method with \( e - \eta \) interaction.

Goodness-of-fit of different models was compared using the NONMEM objective function value OBJ, which is asymptotically \(-2 \log \) likelihood of the data. The difference, \( dOBJ \), between the two models is approximately \( \chi^2 \)-distributed and can be tested for statistic significance (log-likelihood ratio test). A value of \( P < 0.01 \) was considered as significant.

We additionally performed a jack-kneve resampling analysis with 20 replicates to assess the bias of the parameter estimates and to obtain 95% confidence intervals of the parameters.23

Using equation (8), isoboles for half-maximum effect were derived from the typical values of the parameters in the population and also from the individual parameters, which were obtained as Bayesian post hoc estimates.

**Statistical analysis**

All results are expressed as mean (SD) or median (1.–3. quartile) if not stated otherwise. While the statistical analysis for the pharmacodynamic modelling was performed as described above, the measured data were analysed as follows. For each group (alfentanil, physostigmine, and the combination), the maximum effect was tested for statistically significant difference from zero using a one-sided one sample \( t \)-test. Different treatments were compared by post hoc testing using two-sided Student’s \( t \)-tests or Wilcoxon-matched pairs tests, corrected with the Bonferroni procedure. The significance level for these tests was \( P < 0.05 \). Given the sample size of 20 volunteers, a standardized effect size of 0.67 could be detected with a power of 0.8. Statistical analysis was performed with Statistica 6.0 (StatSoft Inc, Tulsa, OK, USA).

**Results**

All of the 20 volunteers completed the study and answered investigators’ questions promptly without showing any
signs of sedation. Heart rate and blood pressure remained unchanged in all groups. Nausea occurred in nine volunteers after administration of physostigmine and in seven volunteers after administration of the combination. Emesis was observed in one volunteer after physostigmine and in one subject after the combination. Neither nausea nor emesis were observed for the placebo and for alfentanil alone.

**Pain rating**

To achieve a pain rating of 6, the average current was increased to 44.3 (SD 22.3) mA in the alfentanil group, to 44.8 (25.6) mA in the physostigmine group, to 48.9 (23.5) mA in the combination group, and to 46.4 (23.3) mA in the control group during the first 15 min of electrical stimulation (NS, t-tests, Bonferroni corrected). Thereafter, the pain ratings decreased significantly, reaching NRS 5 after 15 min in all treatment groups. In the subsequent treatment phase, alfentanil and physostigmine and the combination led to significant analgesic effects (Fig. 2A), that is reduction of pain rating after correction for placebo effects. The maximum effect was 50.4 (SD 22.3) % after alfentanil, 35.4 (20.0) % after physostigmine, and 60.4 (17.1) % after the combination. When compared with physostigmine, the maximum effect was significantly higher both for alfentanil and for the combination, whereas there was no significant difference between alfentanil and the combination. No significant differences in the efficacy of the treatments were observed between male and female volunteers.

**Hyperalgesic areas**

During the first 30 min of electrical stimulation, areas of pinprick-hyperalgesia slightly expanded to a median value of 45 cm$^2$ (8–71 cm$^2$, interquartile range). As observed in earlier studies, there was a considerable variance in the development of secondary hyperalgesia. When corrected for placebo effects, the maximum reduction of the hyperalgesic areas was 53.8 (SD 33.2) % after alfentanil, 47.0 (26.3) % after physostigmine, and 54.8 (33.2) % after the combination. The maximum effect was significantly different from zero in all three groups, but there were no significant differences between the groups. The time courses of the antihyperalgesic effects are shown in Fig. 2B. No significant differences were observed in the antihyperalgesic efficacy of the treatments between male and female volunteers.

**Pharmacodynamic modelling**

The analgesic and the antihyperalgesic effects showed an infra-additive interaction, which was best described by model 2 as defined by the equations (6) and (7), whereas model 1 as defined by equations (3) and (4) was less appropriate (dOBJ=89 for analgesia, dOBJ=10 for antihyperalgesia). Figure 3 shows as an example the fits for both models for the pain rating after alfentanil and physostigmine. For the analgesic effect, the additional parameter $\varepsilon$ did not improve significantly the goodness-of-fit of model 2 (dOBJ=3.0), whereas for the antihyperalgesic effect, the parameter $\varepsilon$ lead to a significant improvement of model 2 (dOBJ=14). Additional parameters for the effect of gender on EC$_{50}$ did not result in a significant improvement of fit (dOBJ<1). The results of the pharmacodynamic modelling are summarized in Tables 1 and 2. There was a marked lag (hysteresis) between the concentration in the central and the effect site compartment for both drugs with $T_{1/2}K_{0}$ between 11 and 35 min.

The jack-knife estimates were in agreement with the estimates obtained from the complete dataset. The measured and predicted time courses with the final model are shown in Figure 4. Figure 5 shows the individual and population isoboles for half-maximum effect. For both analgesic and antihyperalgesic effect, the isoboles indicate
an infra-additive interaction, which was more pronounced for antihyperalgesia. Figure 6 shows the estimated dose–response curves for single application of alfentanil and physostigmine, respectively.

**Discussion**

In this study we investigated the analgesic and antihyperalgesic properties of the cholinesterase inhibitor physostigmine and the opioid alfentanil, alone and in combination, in an experimental pain model in humans. Using pharmacodynamic modelling, we were able to characterize an infra-additive interaction of physostigmine and alfentanil on pain and hyperalgesia in humans.

In recent years medical science has tried to find new ways to treat pain. There has been a tendency away from a monotherapy regime towards combination treatment. This type of therapy had been described as balanced analgesia by Kehlet and colleagues. The idea is to achieve a reduction in dosage with the strongest possible analgesic effect by combining different medications, with different locations of effect to achieve a good overall side-effect ratio. Along with the synergistic effects of opioids and NSAIDs, COX-inhibitors, or acetaminophen the anticholinergic system also seems to play an important part in processing pain. Borovikova and colleagues were able to show that systemic or central application of cholinomimetic substances can cause analgesia. Based on this fact, a cholinergic anti-inflammatory pathway has been described based on a mechanism of neural inhibition of inflammation.

**Table 1** Parameters of the final model (model 2) for the analgesic effect. \( k_{e0} \), elimination rate constant of the effect compartment; EC\(_{50}\), half-maximum effect concentration; \( \gamma \), Hill exponent, describing the steepness of the concentration–effect relationship; \( \epsilon \), interaction parameter; \( \sigma^2 \), intradividual variability; \( \sigma^2 \), interindividual variability; CI, confidence interval

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>( \sigma^2 )</th>
<th>Jack-knife estimate</th>
<th>Jack-knife 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{e0} ) Alfentanil (min(^{-1}))</td>
<td>0.040</td>
<td>0.002</td>
<td>0.051</td>
<td>0.039</td>
<td>0.034–0.046</td>
</tr>
<tr>
<td>( k_{e0} ) Physostigmine (min(^{-1}))</td>
<td>0.020</td>
<td>0.004</td>
<td>0.059</td>
<td>0.020</td>
<td>0.012–0.029</td>
</tr>
<tr>
<td>EC(_{50}) Alfentanil (ng ml(^{-1}))</td>
<td>33.3</td>
<td>1.4</td>
<td>0.013</td>
<td>32.6</td>
<td>28.5–36.7</td>
</tr>
<tr>
<td>EC(_{50}) Physostigmine (ng ml(^{-1}))</td>
<td>25.4</td>
<td>7.7</td>
<td>0.60</td>
<td>24.6</td>
<td>9.0–40.3</td>
</tr>
<tr>
<td>( \gamma ) Alfentanil</td>
<td>2.74</td>
<td>0.60</td>
<td>0.61</td>
<td>2.45</td>
<td>1.22–3.68</td>
</tr>
<tr>
<td>( \gamma ) Physostigmine</td>
<td>1.20</td>
<td>0.24</td>
<td>0.40</td>
<td>1.13</td>
<td>0.61–1.66</td>
</tr>
<tr>
<td>( \epsilon )</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>( \sigma^2 ) Alfentanil</td>
<td>0.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma^2 ) Physostigmine</td>
<td>0.0073</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma^2 ) Alfentanil + Physostigmine</td>
<td>0.016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Parameters of the final model (model 2) for the antihyperalgesic effect. \( k_{e0} \), elimination rate constant of the effect compartment; EC\(_{50}\), half-maximum effect concentration; \( \gamma \), Hill exponent, describing the steepness of the concentration–effect relationship; \( \epsilon \), interaction parameter; \( \sigma^2 \), intradividual variability; \( \sigma^2 \), interindividual variability; CI, confidence interval

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>( \sigma^2 )</th>
<th>Jack-knife estimate</th>
<th>Jack-knife 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{e0} ) Alfentanil (min(^{-1}))</td>
<td>0.056</td>
<td>0.005</td>
<td>0.46</td>
<td>0.054</td>
<td>0.045–0.065</td>
</tr>
<tr>
<td>( k_{e0} ) Physostigmine (min(^{-1}))</td>
<td>0.062</td>
<td>0.012</td>
<td>0.08</td>
<td>0.058</td>
<td>0.031–0.085</td>
</tr>
<tr>
<td>EC(_{50}) Alfentanil (ng ml(^{-1}))</td>
<td>29.9</td>
<td>3.2</td>
<td>0.16</td>
<td>28.7</td>
<td>22.3–35.1</td>
</tr>
<tr>
<td>EC(_{50}) Physostigmine (ng ml(^{-1}))</td>
<td>23.7</td>
<td>5.2</td>
<td>0.32</td>
<td>22.3</td>
<td>10.8–33.3</td>
</tr>
<tr>
<td>( \gamma ) Alfentanil</td>
<td>2.38</td>
<td>0.43</td>
<td>0.13</td>
<td>2.50</td>
<td>1.42–3.58</td>
</tr>
<tr>
<td>( \gamma ) Physostigmine</td>
<td>1.11</td>
<td>0.26</td>
<td>0.17</td>
<td>1.01</td>
<td>0.47–1.56</td>
</tr>
<tr>
<td>( \epsilon )</td>
<td>–0.88</td>
<td>0.23</td>
<td>0.42</td>
<td>–0.85</td>
<td>–1.37 to –0.33</td>
</tr>
<tr>
<td>( \sigma^2 ) Alfentanil</td>
<td>0.046</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma^2 ) Physostigmine</td>
<td>0.043</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma^2 ) Alfentanil + Physostigmine</td>
<td>0.023</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
mediator of the vagus nerve, which inhibits, depending on
the dosage, the production of pro-inflammatory cytokines
of the macrophages including IL-1 beta, II-6 and TNF. These cytokines are suspected of being proalgesic.

The cholinesterase inhibitor physostigmine prevents the
breakdown of the acetylcholine concentration by inhibiting
cholinesterase. Physostigmine crosses the blood–brain
barrier and increases the acetylcholine concentration in the
brain. In 1986, Peterson and colleagues were able to
demonstrate effective analgesia with physostigmine in the
postoperative period. In a clinical study of 2005, Beilin
and colleagues were able to show that by using

---

**Fig 4** Placebo-corrected and normalized analgesic (A–C) and antihyperalgesic (D–F) effects for alfentanil (A, D), physostigmine (B, E), and a combination of both (C, F), respectively. The predictions of the final pharmacodynamic model are drawn with solid lines. Data are expressed as mean ± SE.
physostigmine the use of opiates was significantly reduced and an enhanced analgesic response occurred. The present study aimed to characterize the antinociceptive properties of physostigmine by using a human pain model in volunteers. One advantage of this approach is that the analgesic effect can be clearly distinguished from the antihyperalgesic effect. Previous work has shown, that this model is suitable to test the central component of analgesic and antihyperalgesic effects of opioids and other centrally acting analgesics. We performed a pharmacodynamic modelling to derive concentration–effect relationships for the single drugs and for a combination of the two drugs. This modelling may be helpful for dosage finding in clinical practice.

The single administration of alfentanil led to distinct decreases of analgesic and antihyperalgesic effects. This confirms previous results obtained for alfentanil with our human pain model. As single administration of physostigmine leads to significant decreases in pain ratings and areas of hyperalgesia, physostigmine can be classified as analgesic and antihyperalgesic agent.

When combining both substances, pain ratings and the areas of hyperalgesia were markedly reduced. However, the analgesic and antihyperalgesic effect of the combination was not significantly different from the effects achieved with alfentanil alone. A ceiling effect can be excluded as the maximum effect with one single drug was only about 50%. It must be mentioned that the dosing in the present study was different from that in other interaction studies. Typically, one uses dose combinations such as $D_A$, $D_B$, and $D_A/2+D_B/2$ and tests whether these doses produce the same effect. Another approach is to determine different dose combinations which produce the same effect (for example by using a feedback system), and to construct the isobole from these combinations. In the present study, the dose combinations $D_A$, $D_B$, and $D_A+D_B$ did not allow classical isobolographic analysis. We therefore performed a pharmacokinetic–pharmacodynamic modelling to identify the type of interaction. The isoboles resulted as a visualization of the estimated model, showing an infra-additive interaction for both analgesia and antihyperalgesia (Fig. 5). The infra-additivity of the medications was more pronounced for hyperalgesia than for analgesia.
Infra-additive interaction can be a result of two drugs acting at the same site so that a competitive antagonism occurs. For alfentanil and physostigmine, however, a common receptor can be ruled out, and the interaction might be based on common second messenger pathways. The analgesic effect of opioids is transmitted through µ- and κ-receptors which block the adenylate cyclase via a G-protein. This G-protein-induced block of the intercellular adenylate cyclase can also happen by activating M-receptors through acetylcholine. The strictly infra-additive effect of both physostigmine and alfentanil may be based on an ‘intracellular competitive antagonism’ at the end of the activation cascade of a G-protein. It was possible to show in animal experiments that by stimulating muscarine M2- and M4-receptors on peripheral nerve endings distinctive antinociceptive effects occurred. In subsequent studies it was possible to show that M2- and M4-receptors were also responsible for the antinociceptive effect in major brain regions. Therefore, it can be assumed that opioids and parasympathomimetics, which have a final segment connected by a joint G-protein, lead to an antinociceptive effect are subject to a competitive inhibition.

We found that the interaction was best described by a model which was somehow different from the often used model defined by equations (3) and (4). In the present study, this model 1 was inferior to the alternative model 2 [equations (6) and (7)] because the steepness of the concentration–effect curves of alfentanil and physostigmine were markedly different, whereas model 1 assumes identical γ for both drugs. However, model 2 is less useful if one wants to describe supra-additive interactions with a concave isobole, because for model 2 the shape of the isobole is typically convex if γ>1 which was found for many drugs.

Sex differences in analgesic response to opioid therapy have received increasing attention in recent years, and especially µ- and κ-receptor agonists were found to produce sexually dimorphic analgesia in humans. In the present study, however, no differences were observed in the analgesic or antihyperalgesic response between female and male volunteers.

In conclusion, this study demonstrated distinct effects of physostigmine and an opioid on pain and hyperalgesia in a human pain model. Although an infra-additive interaction was found, the beneficial effects of physostigmine on opioid-induced side effects such as delayed return of bowel function or cognitive dysfunction, may justify the combination of alfentanil and physostigmine for a balanced analgesia regimen in different pain states containing both acute nociceptor pain and hyperalgesia.

Supplementary data
The appendices can be found as supplementary data in British Journal of Anaesthesia online.

Funding
This work was funded by a grant from Dr Koehler Chemie GmbH, Alsbach-Haehnlein, Germany.

References
10 Chiari AI, Eisenach JC. Intrathecal adenosine: interactions with spinal clonidine and neostigmine in rat models of acute nociception and postoperative hypersensitivity. Anesthesiology 1999; 90: 1413–21
17 Weidner C, Schmelz M, Schmidt R, Hansson B, Handwerker HO, Torebjörk HE. Functional attributes discriminating mecha-


