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The Respiratory Effects of the Cytokine Regulating Agent HP 228 Alone and in Combination with Morphine in Human Volunteers

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WEINGER, M. B., S. R. CHAPLAN, B. E. GIRTEN AND F. L. POWELL. *The respiratory effects of the cytokine regulating agent HP 228 alone and in combination with morphine in human volunteers.* PHARMACOL BIOCHEM BEHAV **59**(3) 759–766, 1998.—HP 228 is a synthetic heptapeptide analog of alpha-MSH that attenuates the production and release of inflammatory cytokines. The purpose of this study was to define HP 228's effects, alone and in combination with morphine, on resting ventilation and the ventilatory response to hypoxia and hypercarbia. Six healthy nonsmoking young adult males completed the four-session experiment. Subjects first underwent an initial training session. During subsequent sessions, each subject was tested for the respiratory effects of intravenous HP 228 (30 μg/kg), morphine (0.15 mg/kg), or HP 228 (30 μg/kg) plus morphine (0.15 mg/kg) in a double-blind placebo-controlled randomized balanced within-subjects experimental design. Sessions began with baseline measurement of resting ventilation, oxygen consumption, the isocapnic hypoxic ventilatory response (HVR), and normoxic hypercapnic ventilatory response (HCVR). A second set of respiratory measurements were obtained 10 min after completion of HP 228 or placebo infusion. Morphine or placebo was then administered and ventilatory responses were determined 15 and 40 min postinfusion. HP 228 produced cutaneous flushing, but had no significant effect on respiration or hemodynamics. Morphine significantly decreased metabolism, resting ventilation, and hypoxic and hypercarbic ventilatory responsiveness, independent of prior HP 228 administration. A seventh subject experienced a significant cardiac arrhythmia upon exposure to hypoxia after receiving both HP 228 and morphine and was withdrawn from further study. In conclusion, in this early Phase I clinical trial, HP 228 was found to neither depress ventilation nor augment morphine-induced respiratory depression in healthy young males. © 1998 Elsevier Science Inc.

Cytokines Antiinflammatory agents Analgesics; opioid Morphine; adverse effects
Respiration; drug effects Ventilatory control Chemoreceptors; drug effects Respiration; physiology

HP 228, one of a new class of drugs called "cytokine regulating agents," is a potent, synthetic, enzymatically stable heptapeptide analog of alpha-melanocyte stimulating hormone (α-MSH), which also shares appreciable sequence homology with adrenocorticotrophic hormone (ACTH) (11). HP 228, like α-MSH and ACTH, binds to melanocortin receptors causing an increase in intracellular adenylate cylase activity and melanin production (6). One of HP 228's primary in vivo pharmacological effects appears to be the attenuation of the production and release of inflammatory (and antiinflammatory) cytokines such as tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), interleukin-6, and interleukin-10 (7). In animal studies, HP 228 exhibited a broad spectrum of antiinflammatory, analgesic, and antipyretic actions (5). HP

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228 was an effective analgesic in standard rodent models of inflammatory pain such as carrageenan paw swelling and IL-1β hyperalgesia (8,9,15). HP 228 was also analgesic in an incisional pain model (14) and, when used in combination with morphine, HP 228 modestly augmented morphine-induced analgesia (unpublished data). Although the respiratory effects of HP 228 have not yet been carefully elucidated, in preliminary experiments in rabbits, HP 228 antagonized morphine-induced decreases in respiratory rate (unpublished observations). If HP 228 produces analgesia in humans without significant side effects, including respiratory depression, then it could be useful in the management of acute, and possibly chronic, pain. The purpose of this Phase I human volunteer study was: 1) to define the effects of HP 228 on resting ventilation and the ventilatory response to oxygen and carbon dioxide; and 2) to investigate the effect of HP 228 on morphine-induced respiratory depression. Based on animal data, it was hypothesized that HP 228 would not affect baseline ventilation but would attenuate morphine-induced ventilatory depression.

METHOD

Subjects

Eight healthy nonsmoking adult males signed written informed consent to participate in this study, which was approved by the UCSD Human Subjects Program Institutional Review Board. Subjects were explicitly excluded from participation if they had any clinically significant medical condition, a history of smoking, a currently active or recently treated infection, recent regular use of any medications, a history of substance abuse, or a history of allergy or hypersensitivity to any medication. Subjects abstained from taking any aspirin, aspirin-containing, or nonsteroidal antiinflammatory drugs within the 72 h prior to each experimental session. Subjects also did not eat or drink anything except clear liquids after midnight the day prior to each experiment (including no caffeinated beverages the morning of the study). Subjects were not allowed to drive themselves home after receiving study drugs. All experiments were conducted in a single laboratory room at the Thornton Hospital of the UCSD Medical Center between August and October of 1995.

Drugs

At the time this study was initiated, HP 228 had only been administered to about 30 human volunteers. Early Phase I dose-finding studies suggested that doses less than 200 μg/kg were devoid of serious side effects. Therefore, a dose of 100 μg/kg was chosen for the present study based upon both preliminary human and animal experiments that sugggested this dose would be safe and would produce significant analgesia at the time of peak effect [unpublished Phase I clinical data on file, Houghton Pharmaceuticals, Inc. (now Trega Biosciences), La Jolla, CA]. HP 228 was administered intravenously over 10 min in a volume 10 ml. Morphine sulfate (MS) was admininistered intravenously over 5 min in a dose of 0.15 mg/kg in 10 ml. This dose has been shown to produce significant respiratory depression (4,20) and is an analgesic in opioid-naive subjects. All drugs, prepared in a sterile fashion by a research pharmacist, were adminstered via an intravenous catheter by an anesthesiologist in a double-blind fashion.

Experimental Protocol

Each subject was studied on four occasions at 5–10-day intervals using a double-blind placebo-controlled randomized Latin squares within-subject's experimental design. An initial training session was necessary to familiarize the subjects with the experimental apparatus, and to establish the necessary inspired gas concentrations to obtain optimal stable hypoxic and hypercapnic ventilatory responses. During the three subsequent experimental sessions, each subject was tested for the respiratory effects of intravenous HP 228, morphine, or HP 228 plus morphine in separate sessions (Table 1).

TABLE 1
ALLOCATION OF SUBJECTS TO EXPERIMENTAL TREATMENT GROUPS

	Session 1	Session 2	Session 3	Session 4
Subject A	training	HP/MS	PL/MS	HP/PL
Subject B	training	PL/MS	HP/PL	HP/MS
Subject C	training	HP/PL	HP/MS	PL/MS
Subject D	training	HP/MS	PL/MS	HP/PL
Subject E	training	PL/MS	HP/PL	HP/MS
Subject F	training	HP/PL	HP/MS	PL/MS

HP/MS—HP 228 followed by morphine sulfate.
PL/MS—Placebo followed by morphine sulfate.
HP/PL—HP 228 followed by placebo.

A 115-min protocol (Table 2) was employed that included four ventilatory response measurements (of 10–15 min duration each, described below) made under three conditions: baseline (T1; prior to pretreatment drug); postpretreatment (T2; after HP 228 or placebo); and two posttreatment measurements, beginning 15 min (T3) and 40 min (T4) after initiation of intravenous morphine or placebo administration. At each of the four experimental time points, resting ventilatory measurements including end-tidal PCO_2 ($P_{ET}CO_2$), respiratory rate (f_R), tidal volume (V_T), and pulse oximeter saturation (S_pO_2) were obtained. Then, hypoxic ventilatory response (HVR), hypercapnic ventilatory response (HCVR), and oxygen consumption were measured (see below). In addition, heart rate and blood pressure were recorded regularly throughout each experiment.

Subjects were studied in a semirecumbent position, listening to music of their choice via headphones. If their eyes were closed, they were questioned by the observer regularly to insure they did not fall asleep. At the start of each session, subjects were acclimatized to the measurement apparatus for 10

TABLE 2
TIME COURSE OF EXPERIMENTAL INTERVENTIONS

Time (min)	Test
0	Start room air baseline incl. metabolic rate
10	Begin baseline HVR-HCVR measurements (T1)
25	Pretreatment with HP 228 or placebo (over 10 min)
45	Begin postpretreatment HVR-HCVR and metabolic rate measurements (T2)
60	Treatment with morphine or placebo (over 5 min)
75	Begin 15 min posttreatment HVR-HCVR and metabolic rate measurements (T3)
100	Begin 40 min posttreatment HVR-HCVR and metabolic rate measurements (T4)
115	Session complete

min by breathing room air (21% O_2, 0% CO_2) through a mouthpiece. From 10–25 min, baseline ventilatory responses [respiratory rate, tidal volume, end-tidal PO_2, PCO_2, and estimates of arterial oxygen saturation from a pulse oximeter (S_pO_2)] were measured at 5-min intervals. At 25 min, the subject was allowed to come off the mouthpiece, and the pretreatment drug was administered (HP 228 or placebo). The subject was then reconnected to the respiratory apparatus and baseline measurements were obtained for 10 min. Beginning at 40 min (10 min after completion of the HP 228 or placebo infusion), the second set ventilatory response measurements were obtained. The metabolic rate was also measured at this time.

At 60 min, the subject was allowed to remove the mouthpiece again and morphine or placebo was administered. The subject then breathed room air for 15 min, during which time ventilatory parameters and metabolic rate were measured. At 75 min (15 min posttreatment), the third ventilatory response measurement was obtained. A fourth ventilatory response measurement was obtained at 100 min (40 min posttreatment).

An anethesiologist continuously monitored the subject's behavior, ECG, vital signs, and ventilatory status throughout each experiment. The effects of HP 228 on other behavioral effects of morphine were assessed by asking subjects to complete 10-point visual analog scales (VAS) indicating the magnitude of any pruritus, nausea, or sedation. Subjects completed the VAS immediately before initiation and upon conclusion of each experiment in the series.

Ventilatory Measurements

Expired ventilatory flow was measured with a pneumotachograph in subjects wearing a noseclip and breathing through a mouthpiece connected to a nonrebreathing valve. A three-way valve directed either room air or a ventilatory circuit delivering $O_2/CO_2/N_2$ mixtures to the inspiratory port of the nonrebreathing valve (19). End-tidal PO_2 and PCO_2 were continuously measured with a mass spectrometer (Perkin–Elmer MGA 110), and arterial O_2 saturation (S_pO_2) was continuously measured with a finger pulse oximeter (Physiocontrol Lifestat 1600). These signals were recorded on a chart recorder and digitized on line for subsequent computer analysis of inpired minute ventilation (V_I)), tidal volume (V_T), respiratory frequency (f_R), and peak end-tidal PCO_2 ($P_{ET}CO_2$). Metabolic rate (V_{O2} and V_{CO2}) was measured by analyzing mixed-expired O_2 and CO_2 collected for 2–3 min into a Douglas bag from the expiratory limb of the ventilation circuit. Temperature was measured using a gently applied infrared noninvasive tympanic thermistor (Thermoscan™ HM-2, San Diego, CA). Blood pressure and heart rate were monitored every 5 min with an automated noninvasive blood pressure device (Critikon DINAMAP™, Tampa, FL).

Ventilatory response measurements began by establishing a baseline while the subjects breathed room air for 5–10 min. Then, the three-way valve was turned to connect the subjects to the ventilatory circuit and the hypercapnic ventilatory response (HCVR) was measured. If S_pO_2 was less than 98%, for example with morphine-induced ventilatory depression, then the inspired O_2 was increased to 30% to remove any O_2-sensitive ventilatory drive and establish the first point of the HCVR. Two more points of the HCVR were measured by increasing inspired CO_2 as necessary to elevate $P_{ET}CO_2$ by 4 Torr for 3 min, and approximately 4 Torr more for 2 additional min. Next, the isocapnic hypoxic ventilatory response (HVR) was measured by lowering inspired O_2 as necessary to hold S_pO_2 at 80–85% for 5 min. $P_{ET}CO_2$ values were maintained at 4 Torr above the normoxic baseline value by adjusting inspired CO_2; this CO_2 level was chosen to match the hypercarbia expected with morphine-induced ventilatory depression (4). The entire HCVR and HVR protocol took less than 15 min after the normoxic baseline had been established.

Data Analysis

V_I was calculated as the product of V_T and f_R, measured by integrating the digitized inspiratory flow data and counting $P_{ET}CO_2$ peaks from the digitized mass spectrometer recordings, respectively, with a PC using an in-house software package developed by colleagues at UCSD. Only data collected between 3 and 5 min of a stable stimulus level were used for analysis. V_I was normalized to body mass for analysis and ventilatory response calculations. The HCVR was calculated as the increase in V_I per Torr increase in $P_{ET}CO_2$ measured between the first and second levels of normoxic hypercapnia (i.e., between 4 and 8 Torr above normoxic baseline $P_{ET}CO_2$). The isocapnic HVR was calculated as the increase in V_I per % S_pO_2 measured between the first normoxic hypercapnic level (S_pO_2 of 100%) and the final hypoxic level (target of 85%). Both measures of ventilatory responsiveness are linear over the range studied (17). V_{O2} and V_{CO2} were calculated from V_I measured during mixed expired gas collection in a bag, and the difference between average inspired and mixed expired gas concentrations.

Ventilatory and hemodynamic data are presented and analyzed in terms of the difference between the first baseline measurement (T1) and each subsequent treatment in a given session (see Table 2). Thus, three pieces of data were calculated for each dependent variable in a session: 1) baseline value minus the value after pretreatment with HP 228 or placebo (T2), 2) baseline value minus the value 15 min posttreatment with morphine or placebo (T3), and 3) baseline value minus the value 40 min posttreatment with morphine or placebo (T4).

Statistical significance was determined using repeated-measures ANOVA followed by Newman–Keuls a posteriori tests (22). Significant effects of treatment at each measurement period (T2, T3, and T4) used the difference from baseline, as described above. For each treatment, differences in raw values of the dependent variables were assessed between time points. A $p < 0.05$ was considered statistically significant, and all results are reported as means ± standard errors of the mean (SEM).

RESULTS

Two subjects started the experiment but did not finish: subject #6 was asked to withdraw because he had an unusually low resting respiratory rate (≤3/min) during the training session, and subject #2 was withdrawn after the second session (first experimental session) because of an adverse cardiovascular event. In this latter subject, significant bradycardia and frequent unifocal ventricular ectopy occurred after exposure to hypoxia (S_pO_2 <85%) following treatment with intravenous HP 228 and morphine. Upon completion of the last set of respiratory measurements, associated hypotension and concurrent nausea and light-headedness necessitated treatment with atropine, ephedrine, and intravenous fluids. Although the hypotension resolved rapidly, the ventricular ectopy and his subjective symptoms persisted for several hours.

Six of seven subjects experienced nausea and/or lightheadedness following morphine administration. One subject had persistent nausea and anorexia overnight after his first ex-

posure to morphine. Following HP 228 administration, four subjects exhibited facial flushing and two subjects had transient penile erections. None of these side effects appeared to interfere with the ventilatory measurements (which were obtained while the subject remained motionless in the semirecumbent position).

Baseline Measurements (Prior to Study Drug Administration)

A systematic difference was observed between the first training session and all subsequent experimental sessions (Table 3). Ventilation and CO_2 production during the air breathing baseline in the three experimental sessions (sessions 2–4) were greater than the average of the baseline measurements in session 1 (training session) as reflected in increased V_I/kg and decreased $P_{ET}CO_2$. R was also greater during the three experimental sessions vs. the first (training) session, indicating hyperventilation at the start of the sessions in which drugs were administered. The differences in baseline ventilatory values between the training and experimental sessions disappeared when the subjects were exposed to hypoxia for the first time (T1) during each session. Also, the degree of hypoxia during T1 was slightly less during the training session vs. the experimental sessions (S_pO_2 86.7 ± 1.3 vs. 83.9 ± 0.9; $p < 0.05$) because inspired gases could be adjusted more accurately to obtain the target levels during the experimental sessions after individual values were established in the training session.

Initial baseline heart rate and systolic blood pressure (T1 measurement) were not different between the training and experimental sessions (Table 3). During the T1 measurement, exposure to hypoxia and mild hypercapnia (see the Method section) resulted in a significant increase in respiratory rate, tidal volume, minute ventilation, and heart rate. There were no significant differences between the three experimental sessions in the air-breathing baseline (predrug) ventilation or hemodynamic values.

The Respiratory and Hemodynamic Effects of Morphine and HP 228

V_I decreased significantly (over 40 l/min-kg) 40 min after morphine treatment, independent of prior HP 228 pretreatment (Fig. 1A). $P_{ET}CO_2$ increased an average of 4 ± 2 Torr in both morphine treatment groups (Fig. 1B), although the effect was limited by a significant decrease in metabolic rate after morphine (Fig. 1C). V_{CO2} decreased from 250 ± 25 to 180 ± 26 ml/min, and this effect was independent of HP 228 pretreatment. There were no significant effects of treatment on blood pressure (Table 4). In contrast, heart rate decreased through the session, to a somewhat greater extent in the two morphine-treated groups.

Morphine significantly decreased the HVR independent of prior HP 228 administration at 40 min posttreatment (Fig. 2A). The effect at 15-min posttreatment was of essentially the same magnitude, and almost attained significance ($p < 0.06$). The HCVR was also decreased by morphine independent of HP 228 (Fig. 2B), but this effect did not attain statistical significance at either the 15-min ($p < 0.10$) or the 40-min ($p < 0.08$) posttreatment measurement periods.

HP 228 alone had no significant effect on any of the hemodynamic or ventilatory variables measured (see Fig. 1 and Table 4). Some individuals showed increased HVR and HCVR after HP 228, but the group trend did not attain statistical significance (Fig. 2).

DISCUSSION

Experimental Issues

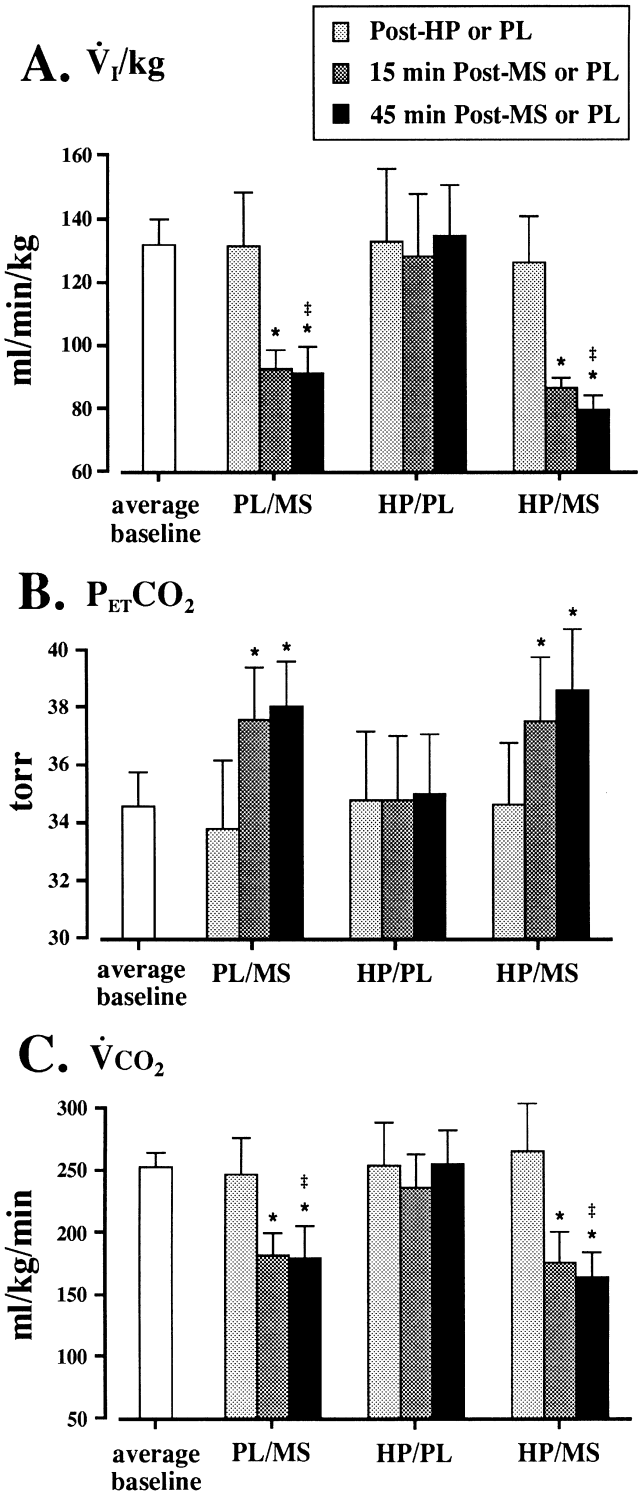
The group of normal healthy male volunteer subjects chosen at random for this study demonstrated an appreciable range of resting ventilation as well as ventilatory responses to O_2 and CO_2. It might have been possible to reduce this variability and, thus, to maximize the chances of detecting changes

TABLE 3

BASELINE VALUES FROM THE FIRST SET OF MEASUREMENTS (PREDRUG) IN THE TRAINING AND THE THREE EXPERIMENTAL SESSIONS

	Normoxic Baseline		During Hypoxia	
Variable	Training	Experimental	Training	Experimental
$P_{ET}CO_2$ (Torr)	40.9 ± 1.3	34.6 ± 1.2*	44.2 ± 1.0‡	42.3 ± 0.7‡
S_aO_2 (%)	96.3 ± 0.3	97.2 ± 0.3	86.7 ± 1.3‡	83.9 ± 0.6*‡
f_R (breaths/min)	9.8 ± 1.5	12.4 ± 0.9	15.5 ± 2.1§	18.1 ± 2.4§
V_T/kg (ml/kg)	9.8 ± 1.2	11.0 ± 0.7	24.2 ± 2.8‡	24.6 ± 2.4‡
V_I/kg (ml/kg/min)	88.7 ± 9.9	132.4 ± 8.0†	362.9 ± 53.6‡	442.0 ± 59.1‡
HR (beats/min)	69 ± 3	69 ± 3	85 ± 2‡	85 ± 2‡
Systolic BP (Torr)	127 ± 2	127 ± 3	134 ± 1	134 ± 1
HVR/kg	21.9 ± 3.5	20.7 ± 3.8		
HCVR/kg	37.5 ± 12.5	34.9 ± 7.5		
VO_2	274.5 ± 40.9	275.5 ± 20.3		
VCO_2	197.8 ± 18.2	253.6 ± 11.9*		
R	0.75 ± 0.05	0.95 ± 0.03†		

*$p < 0.05$ for training vs. experimental.
†$p < 0.01$ for training vs. experimental.
‡$p < 0.01$ for normoxia vs. hypoxia.
§$p < 0.05$ for normoxia vs. hypoxia.



in ventilatory responses by a priori selecting (i.e., prescreening) a group with high ventilatory responses. However, because the purpose of this study was to evaluate the effects of a new drug on humans in the population at large, we avoided this prescreening approach, except for rejecting the one subject with an extremely low respiratory frequency. The respiratory response variability observed was typical of other studies from our laboratory using the same approach.

The variability in HVR of this population was similar to that from other groups of normal volunteers studied in our laboratory. Using data from an earlier study on changes in the HVR during 25 min of sustained hypoxia (Elliott, Weinger, and Powell, in preparation), we performed a power analysis to estimate the number of subjects needed to reveal a significant effect in the current study. The magnitude of decline in the HVR during sustained hypoxia, termed hypoxic ventilatory decline, is similar to, or less than, the observed decrease in HVR with morphine (1,19). For eight subjects, we calculated that the power of detecting a significant decrease in hypoxic ventilatory decline, which equaled 1.5 times the standard deviation of the mean decline, was 75%. This magnitude of change we chose as significant corresponded to about 90% of the average hypoxic ventilatory decline. We chose such a conservative design, which tested for almost complete reversal of the effects of morphine by HP 228, because of the experimental nature of the drug. With this design, we conclude that with eight normal subjects there was less than a 25% chance for our experimental design to "miss" a significant reversal of morphine-induced depression of the HVR by HP 228.

Two factors made the data from the first session undesirable for use as each individual subject's baseline respiratory data. First, there was appreciable variability between measurements in the first session, when the appropriate inspired gas mixtures to attain specific levels of S_pO_2 and $P_{ET}CO_2$ were being established by trial and error. Second, and more importantly, there was a systematic difference in the resting ventilation and metabolic rate observed between the first and all subsequent sessions. Both resting ventilation and metabolic rate were consistently lower in each subject in their first session compared with the three subsequent experimental sessions. This was opposite the direction predicted if the subjects acclimitized to the experimental setup upon repeated exposure (i.e., a "training" effect), and suggests that the subjects were more anxious and excited on the days they were to receive the intravenous study drug injections. Consequently, air

FIG. 1. The histogram depicts the average values (± SEM) for inspired ventilation (A; V_I/kg in ml/min/kg), end-tidal carbon dioxide tension (B; $P_{ET}CO_2$ in Torr), and carbon dioxide production (C; V_{CO2} in ml/kg/min) for subjects in each treatment group (n = 6/group) compared with the average (n = 18) predrug baseline (T1 in Table 2) across all three experimental sessions. The three treatment groups are: placebo followed by morphine (PL/MS), HP 228 followed by placebo (HP/PL), and HP 228 followed by morphine (HP/MS). The average baseline (T1) values across all treatment groups is displayed for comparison purposes. The three measurement periods are post-HP or PL (T2), 15 min post-MS or PL (T3), or 45 min post-MS or PL (T4). The effect of morphine treatment was significantly different from baseline values (*$p < 0.05$) independent of prior treatment with HP 228 for all three ventilatory measures. In addition, for V_I/kg (A) and V_{CO2} (C), but not for $P_{ET}CO_2$ (B), there was a significant effect at the 45-min postmorphine time point (‡$p < 0.05$) compared with the HP/PL group.

TABLE 4
EFFECTS OF MORPHINE (MS) AND HP 228 (HP) VS. PLACEBO (PL) ON SYSTOLIC BLOOD PRESSURE AND HEART RATE

	Post-HP/PL	15 min Post-MS/PL	45 min Post-MS/PL
Systolic BP (Torr)			
PL/MS	122 ± 4	123 ± 4	122 ± 5
HP/PL	128 ± 2	128 ± 2	131 ± 3
HP/MS	129 ± 2	133 ± 5	130 ± 4
Heart Rate (beats/min)			
PL/MS	67 ± 6	68 ± 7	63 ± 7
HP/PL	64 ± 3	63 ± 3	59 ± 4
HP/MS	66 ± 4	62 ± 5	59 ± 4

breathing baseline data from each experimental drug session was used in the analysis, instead of measurements from the first session, as was originally intended.

The Respiratory Effects of HP 228 and Morphine

Morphine's depressant effects on ventilation, ventilatory responses to CO_2 and O_2, and metabolism in this study were similar to those reported in the literature (4,12,13,21). Ventilation is decreased proportionally more than metabolism, resulting in CO_2 retention. This reflects an increase in the arterial PCO_2 "set point" or the intercept of the ventilatory response to CO_2, as observed in our $P_{ET}CO_2$ data (Fig. 1B). Other measures of ventilatory control reflexes include the HCVR, which is the "gain," or slope, of the ventilatory response to CO_2. We failed to observe a significant decrease in HCVR, consistent with other studies using steady-state CO_2 exposures (4). In contrast, the effect of morphine on the HCVR when measured by the rebreathing method is generally greater, presumably because the rebreathing method may not allow the direct and indirect effects of cerebral blood flow on central chemoreceptors to stabilize (3,4). Therefore, the primary effect of morphine on the ventilatory response to CO_2 is a change in "set point," i.e., normoxic $PaCO_2$ and $P_{ET}CO_2$. In contrast, morphine decreases the slope of the hypoxic ventilatory response ($HVR = -\triangle V_I/\triangle S_aO_2$) in humans (1,21).

The results do not support the hypothesis that HP 228 attenuates morphine-induced ventilatory depression, at least in the population studied under these conditions. Although there appeared to be a trend toward an HP 228-induced enhancement of $P_{ET}CO_2$, due to the small number of subjects and the large degree of intersubject variability (as discussed above), this effect did not attain statistical significance. Although there is insufficient evidence from this study to suggest that HP 228 could reduce morphine-induced depression of the ventilatory response to CO_2, this hypothesis may warrant additional study. Although such an effect may not be large, it could attain significance in a more powerful study (i.e., larger sample size) or in a selected population subgroup. The number of subjects enrolled in this study was limited by the extensive nature of the repeated-measures protocol, the willingness of volunteers to participate, and the cost of the study.

Similar qualifications apply to the interpretation of the data on the ventilatory response to hypoxia. The results suggest a stimulating effect of HP 228 on the HVR, but it was not significant. Our measure of HVR was adequate for detecting

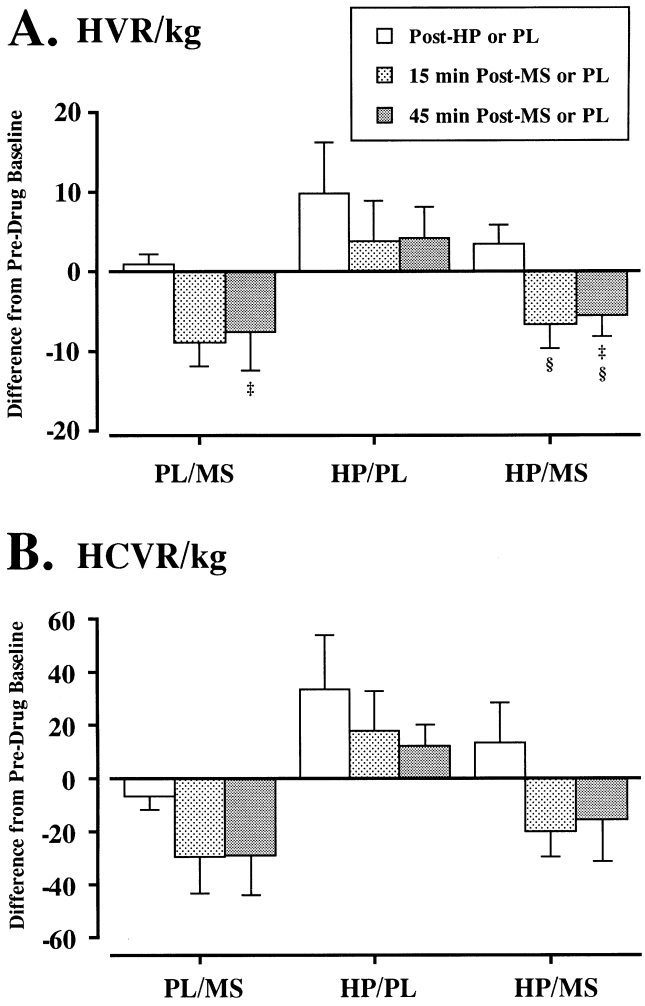


FIG. 2. The histogram depicts the averages (and SEM) for all subjects ($n = 6$) for the hypoxic ventilatory response (A; HVR/kg expressed in ml/kg/min/%SaO_2) and for the hypercarbic ventilatory response (B; HCVR/kg expressed in ml/kg/min/Torr) as the difference from the predrug baseline (T1) during each experimental session. The three experimental measurement periods (T2–T4) and the three treatments are the same as described in Fig. 1. Morphine treatment significantly depressed HVR, independent of prior treatment with HP 228, when compared with the HP/PL group (‡$p < 0.05$). Additionally, in the HP/MS group, morphine treatment significantly depressed HVR compared with the last premorphine (T2) measurement (§$p < 0.05$). A similar pattern of effect of HCVR did not attain statistical significance ($p < 0.10$).

significant effects of a drug, as evidenced by the significant decrease in HVR we observed with morphine, similar to other investigators using the same method to quantify HVR and similar morphine IV doses (1,19). A significant effect of HP 228 on morphine-induced depression of the HVR might be demonstrated in a larger group of subjects. Alternatively, a significant effect of HP 228 on morphine-induced depression of HVR might be observed with a deeper degree of hypoxia, but this was not considered prudent in this clinical trial of an investigational drug.

Side-Effects of HP 228

HP 228 has been shown in animal studies to be devoid of effects on body weight, gastrointestinal transit, gastric fluid volume, hydrogen ion cencentration, pepsin activity, or coagulation. After acute intravenous doses, deaths in rats were not observed until doses of 75 mg/kg. In contrast, based upon early Phase I dose-finding safety studies, human doses less than 200 μg/kg were devoid of serious side effects.

An episode of hypoxia-associated bradycardia, ventricular ectopy, and nausea occurred in a single subject during his first experimental session after receiving both HP 228 and morphine. This subject had been exposed to hypoxia, not only during a prior training session but also on several previous occasions for other ventilatory studies, without a similar reaction. Thus, one may speculate that the symptoms were due to the interaction of the study drugs with hypoxia. However, it cannot be determined whether the effect was due to HP 228, morphine, prolonged fasting, or a combination of these factors. Because of the potential severity of the reaction, it was elected to withdraw the subject from the study, and thus, a definitive answer, which might have been available after two subsequent experimental sessions (i.e., HP/PL and PL/MS), was not obtained. However, it is hypothesized that this reaction was due to the interaction of morphine and hypoxia because: 1) the symptoms appeared to be vagal in etiology; 2) morphine is known to have vagotonic activity; and 3) HP 228 has not previously, in either animals or humans, produced vagal symptomatology. There were no changes in cardiovascular variables at HP 228 doses up to 5 mg/kg in rats or 1 mg/kg in dogs (unpublished data). Nevertheless, this event was considered clinically significant and was reported to the FDA. Further studies will be required to ascertain whether HP 228, with or without opiates, augments the vagotonic effects of hypoxia exposure.

CONCLUSIONS

In the last few years there has been increasing interest in the role of inflammatory cytokines in tissue injury, infection, and postoperative outcome (18). Increased plasma levels of cytokines such as IL-6, IL-1β, and TNFα have been observed after surgery (2,10) and are believed to modulate the neuroendocrine response to surgery (18). In fact, a recent animal study has led to the speculation that the failure of high-dose opiates to block fully the neuroendocrine response to surgical stimulation is due to their inability to attenuate surgically mediated release of cytokines, which then activate neuroendocrine systems (16). If this hypothesis is borne out in subsequent research, then HP 228 may represent the first of a class of drugs that could have a profound impact on clinical management in the perioperative period.

Consistent with their mediation of inflammatory, immunological, and neurohumeral responses to surgery, cytokines also appear to play a role in the adverse side effects of cancer chemotherapy such as nausea and vomiting, mucositis, anorexia, fatigue, weight loss, and organ dysfunction. In addition, inappropriate levels of inflammatory cytokines have been associated with cancer progression. Animal studies suggest that HP 228 may be effective at reducing the incidence and severity of these chemotherapy-induced toxicities. Phase I clinical studies are currently underway to ascertain if HP 228 has beneficial effects in patients receiving cancer chemotherapy. Future studies may be warranted to test the effects of HP 228 on the inflammatory and neurohumeral response to surgery, analgesia, and clinical outcome in the perioperative period.

The potential for HP 228 to produce significant analgesia without associated respiratory depression could represent a therapeutic advance in the management of acute and, possibly, chronic pain. Because HP 228 is a peptide, it can only be administered parenterally. Epidural or intrathecal administration may prove to be a viable alternative dosing route. This human volunteer study suggests that HP 228 has no intrinsic respiratory depressant properties and does not affect significantly morphine-induced respiratory depression. Further clinical and preclinical research will be necessary to delineate the pharmacotherapeutic role for HP 228 and other cytokine regulating agents.

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