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Alfentanil potentiates anaesthetic and electroencephalographic responses to ketamine in the rat

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Abstract

The interactions between μ-opioid and *N*-methyl-D-aspartate (NMDA) receptors have important implications for clinical pain management. We recently examined the pharmacokinetics of ketamine in rats following i.v. infusion of ketamine (racemate, 50 mg/kg/5 min) and found increased central nervous system distribution of ketamine in the presence of low constant plasma alfentanil concentrations (~ 50 ng/ml). We now report on the effects of low plasma alfentanil concentrations on the duration of anaesthetic and electroencephalographic (EEG) responses to i.v. infusion of ketamine. Compared to ketamine alone, alfentanil significantly increased both the duration of anaesthesia (by 130%, $P=0.00022$) and the processed EEG power ($\mu V^2/s$) (by 48%, $P=0.0040$). The plasma ketamine concentration producing half-maximal EEG effect was significantly reduced (by 60%, $P<0.0001$) in the presence of alfentanil. The results indicate that low plasma alfentanil concentrations potentiate the anaesthetic and EEG effects produced by ketamine.
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Keywords: Alfentanil; Anaesthesia; Analgesia; EEG (electroencephalogram); Ketamine; Stereoisomer

1. Introduction

Ketamine acts as a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist by binding to the phencyclidine site in the NMDA receptor-ion channel. Clinically, ketamine induces a state of dissociative anaesthesia and analgesia; it also has psychotomimetic properties (Kohrs and Durieux, 1998). Low doses of *rac*-ketamine (hereafter referred to as ketamine) have also been found to exert effective but brief analgesic effects, mediated by non-opioid mechanisms, in clinical studies of ischaemic and postoperative pain states (Maurset et al., 1989). Recently, ketamine was found to exert supraspinal analgesic effects in rats by the activation of descending monoaminergic pathways but, in the presence of peripheral inflammation, it appeared to produce anti-hyperalgesic effects by inhibiting NMDA receptor activation, although its analgesic effects were no longer apparent (Kawamata et al., 2000).

In clinical use, it has been reported that ketamine interacts favourably with opioids, such that low-dose (i.e. non-anaesthetic) infusions of ketamine when combined with opioid administration produce better postoperative pain relief and have an opioid-sparing effect of up to 50% (Schmid et al., 1999). As well, the pre-emptive use of low-dose ketamine has been found to reduce postoperative pain intensity and consumption of concomitant analgesics (Schmid et al., 1999). In other experimental studies, it has been found that the combination of threshold doses of morphine with low doses of 7-chlorokynurenate, an antagonist at the glycine site of the NMDA receptor, produced a synergistic reduction in the wind-up of rat dorsal horn nociceptive neurons that follow repeated C-fibre stimulation (Chapman and Dickenson, 1992). Moreover, in recent behavioral studies, ketamine was found to potentiate the spinally mediated antinociceptive effects of fentanyl (Nadeson et al., 2002). However, investigations examining the interaction between morphine and dizocilpine (MK-801), a noncompetitive NMDA receptor antagonist, found that their co-administration to rats produced only an additive effect in ameliorating hyperalgesic states induced either by chronic nerve injury (Yamamoto and Yaksh, 1992) or carrageenan injection (Yamamoto et al., 1993).

Alfentanil exerts its pharmacological effects by an almost exclusive action at μ-opioid receptors. Its low tissue solubility

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and high potential binding, compared to congeneric opioids, result in a short plasma elimination half-life and small apparent volume of distribution (Bovill, 1987). These properties make it ideally suited to intravenous infusions that maintain constant plasma drug concentrations. In clinical studies of neuropathic pain, alfentanil was reported to produce analgesic effects when plasma alfentanil concentrations were maintained at 50–75 ng/ml by computer-controlled infusions (Leung et al., 2001). Experimental investigations (Cox et al., 1998c) have found an EC_{50} of 24 ng/ml for the antinociceptive effect of alfentanil on tooth pulp evoked potentials in the electroencephalogram (EEG) recordings from rats.

In studies of hyperalgesia induced in normal human subjects, only a simple additive interaction was found between alfentanil and ketamine (Sethna et al., 1998). However, other experimental studies have found that the development of acute tolerance and the rebound hyperalgesia induced by alfentanil were attenuated by ketamine as well as by MK-801 (Kissin et al., 2000). Acute tolerance has been shown to develop rapidly in rats to both the analgesic effects and EEG signal response of alfentanil (Kissin et al., 1996; Cox et al., 1998b,c). NMDA receptor activation appears to play a critical role in the development of both injury induced hyperalgesia as well as opioid tolerance and the associated rebound hyperalgesia (Mao et al., 1995).

In recent work, we examined the pharmacokinetic interaction of ketamine and alfentanil. Following infusion of ketamine to rats (50 mg/kg/5 min), the presence of low background plasma alfentanil concentrations (~ 50 ng/ml, maintained by computer-controlled infusions) increased both the total body clearance and apparent volume of distribution of ketamine (by 16% and 28%, respectively); moreover, the regional distribution coefficients of ketamine into central nervous system tissues were greater (by ~ 100–300%) in the presence of alfentanil (Edwards et al., 2002). In this report, we present complementary pharmacodynamic data on the effects of low background plasma alfentanil concentrations on the anaesthetic and EEG responses to ketamine infusion that could have further favourable implications for pain management.

2. Materials and methods

2.1. Animal preparation

The study was approved by the Animal Care and Ethics Committee of Royal North Shore Hospital.

Adult male Wistar rats (350–400 g, obtained from the Gore Hill Research Laboratories, Sydney, Australia) were housed in groups of four at 21 °C on a 12/12 h light/dark cycle with free access to food and water. Following surgery, they were housed individually.

Recording electrodes made from 0–80 × 3/32 stainless steel mounting screws (Plastics One, Roanoke, VA, USA), soldered to 1.5-cm lengths of IDC computer cable, were implanted under general anaesthesia (pentobarbitone 30 mg/kg, i.p., followed 5 min later by ketamine 45 mg/kg, i.p.). Four holes were made in the skull 2–3 mm on either side of bregma and lambda with a D60 drill bit (Plastics One) held in a pin vise, and the recording electrodes were screwed down to make contact with the dura. A fifth screw midway between bregma and lambda and 3–4 mm lateral to the midline served as an anchor. Heat shrink tubing (4–5-mm height) was placed around the perimeter of the screws and filled with dental acrylic before the electrode ends were soldered to an eight-pin IC socket. The electrical wire and IC socket were subsequently embedded in dental acrylic. Body weight was allowed to return to baseline before vascular cannulation was performed.

Under general anaesthesia (as above), vascular cannulation was performed using silastic laboratory tubing (respectively 0.025″ I.D. × 0.047″ O.D., and 0.020″ I.D. × 0.037″ O.D.) inserted 2.5 cm into the jugular vein and 2.0 cm into the carotid artery. Cannulae were tunneled under the skin, externalized above the neck anterior to the shoulder blades, and filled with a solution containing 6 g polyvinylpyrrolidone (MW 40,000, Sigma-Aldrich, Sydney, Australia) dissolved in 5 ml of 1000 U/ml sodium heparin (Delta West, Perth, Australia). At the conclusion of each surgical procedure, the animals were administered buprenorphine (0.2 mg/kg, s.c.) for pain control and procaine penicillin + benzathine penicillin (standard veterinary preparation, each at 150 mg/kg, 0.1 ml, s.c.) for anti-microbial cover. Experimental studies were carried out 24 h after vascular cannulation.

2.2. Drugs

Ketamine hydrochloride (racemate: Ketalar™, Parke-Davis, Sydney, Australia) was diluted to 20 mg/ml (17.3 mg/ml as base); alfentanil hydrochloride (Rapifen™, AstraZeneca, Sydney, Australia) was diluted to 100 μg/ml (92 μg/ml as base). Both drugs were diluted with deionized water; the alfetanil solution contained 5 U/ml of heparin.

2.3. EEG data acquisition and processing

EEG recordings were taken from a single pair of electrodes positioned contralaterally across the frontal and occipital lobes. The EEG signal was recorded using a Biopac EEG100 amplifier module (Biopac Systems, Santa Barbara, CA, USA) with the gain set to 5000 and the 1–30 Hz band pass filter activated. The amplifier was connected to an analogue-to-digital converter (Biopac Systems); the signal was acquired and processed with a personal computer (Pentium 120 MHz) by Acqknowledge III software (Biopac Systems). The squared transformation of the EEG signal amplitude (μV) derived from the 1–30 Hz filtered EEG signal was used as a surrogate quantitative measure of CNS

activation to relate to plasma drug concentrations (Mandema and Danhof, 1992). A data acquisition integral function was used to determine the area under the curve of the square-transformed EEG signal (power = μV^2) for 10-s epochs over the duration of the recordings. The mean value of power for each 10-s epoch ($\mu V^2/s$) was then determined and subsequently used as the outcome variable.

2.4. Experimental design

The study contained four groups of animals; all studies were performed between 14:00 and 19:00 h. After infusion and sampling lines (75 and 45 cm, respectively) were attached to the indwelling cannulae, the recording cable (2-m length, seven core-shielded electrical cable) was attached to the electrode block and the animals were placed in a study chamber. The animals were then allowed to settle for 30 min before commencement of the study. An initial baseline EEG recording was taken for 10 min, prior to commencing the drug infusions, with the animals maintained in an alert state. Drug infusions were then delivered by a syringe driver (Harvard Apparatus, Model 22) from a gas-tight syringe (Hamilton, 5 ml), and the EEG was recorded for 180 min following commencement of the infusions. When the righting reflex was lost, the animals were removed from the study chamber; a rectal probe was then inserted to monitor body temperature and a heating lamp maintained body temperature. When motor function began to return, the rectal probe was removed and animals were returned to the study chamber.

In *group 1*, ketamine was infused intravenously at a constant rate of 10 mg/kg/min over 5 min. *Group 2* received the same ketamine infusion over 5 min as group 1. The infusion line for ketamine was then removed, and a new infusion line primed with alfentanil was attached. Seven minutes later (i.e. 12 min after the ketamine infusion began), a computer-controlled infusion program (“Stanpump”; Shafer and Gregg, 1992) commenced an infusion of alfentanil that maintained plasma alfentanil concentrations at ~ 50 ng/ml (Edwards and Mather, 2001) for the remaining

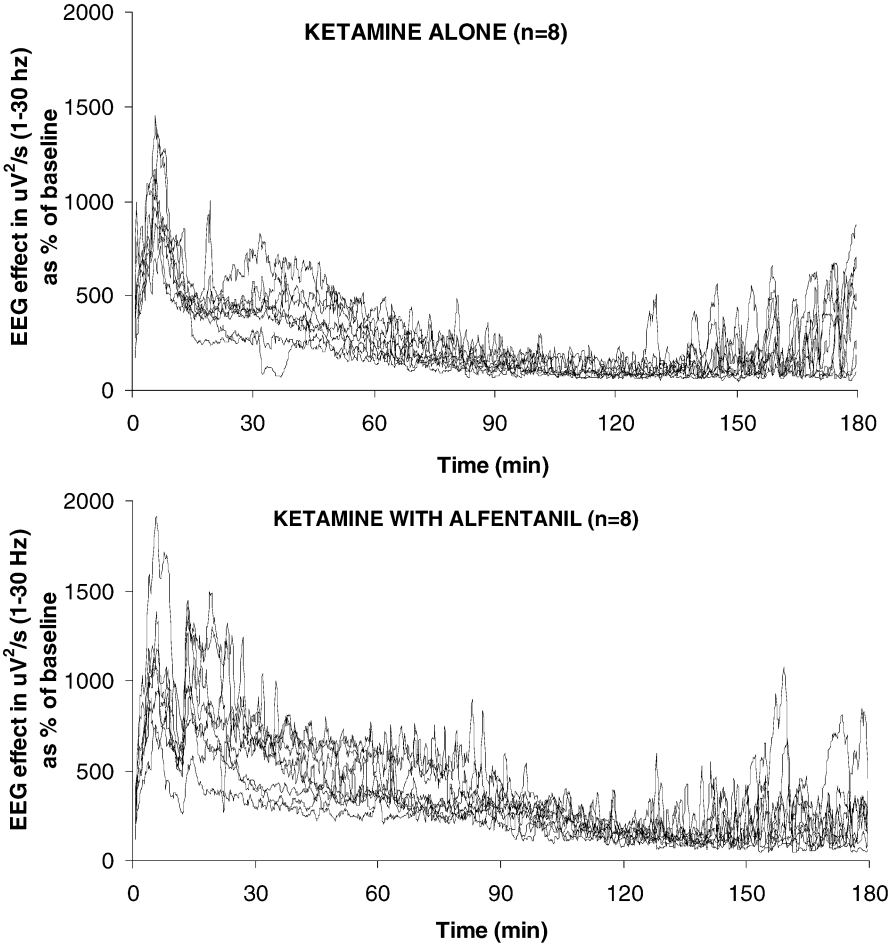

Fig. 1. Processed EEG power data ($\mu V^2/s$) from individual rats expressed as a percentage of the respective value derived from the 10-min baseline recording period in the same animal. Data are presented as the moving average over five consecutive 10-s epochs. Animals in both groups 1 (top panel) and 2 (bottom panel) received ketamine infusions that commenced at 0 min and were delivered at 10 mg/kg/min for 5 min. In Group 2, however, alfentanil infusions delivered by a computer-controlled infusion program (Stanpump) commenced 7 min after ketamine infusions concluded and maintained plasma alfentanil concentrations at ~ 50 ng/ml for the remaining study period.

duration of the study period. In both groups, arterial blood samples (100 μl) were taken before, then at 1.5, 3, 5, 7, 10, 15, 20, 30, 40, 60, 80, 120, 140, 160, and 180 min, and collected into heparinized (50 units) polyethylene microfuge tubes for drug analysis. *Group 3* received only computer-controlled alfentanil infusions that maintained plasma alfentanil concentrations at ~ 50 ng/ml. In *group 4*, EEG recordings were taken from control animals that received neither drug infusion. Each blood sample was replaced with three volumes of 0.9% saline as previously described (Mather et al., 2000). Plasma concentrations of ketamine (summed enantiomers) and alfentanil were determined by previously published techniques (Edwards and Mather, 2001; Edwards et al., 2002).

2.5. Behavioral endpoints for recovery from ketamine-induced anaesthesia

The recovery of animals from ketamine-induced anaesthesia and the associated post-anaesthetic emergence reaction was assessed by measuring the time taken to reach the following behavioral endpoints after commencement of the infusions. First, the return of motor function and coordination was estimated by measuring the times until (i) removal of the thermal rectal probe and return to the chamber, (ii) the return to a sitting posture on four legs, (iii) and the return of coordinated locomotion. Second, recovery from the posthypnotic central-stimulant effects of ketamine was estimated by measuring the time, from the beginning of the infusion, required for the animal to return to a quiescent inactive and immobile state.

2.6. Data analysis

For each animal, the medians of triplicate values for processed EEG power were determined from 30-s periods spanning the time intervals at which the blood samples were taken; these were expressed as percentages of the respective mean value determined for the 10-min baseline recording. Areas under the processed EEG power vs.

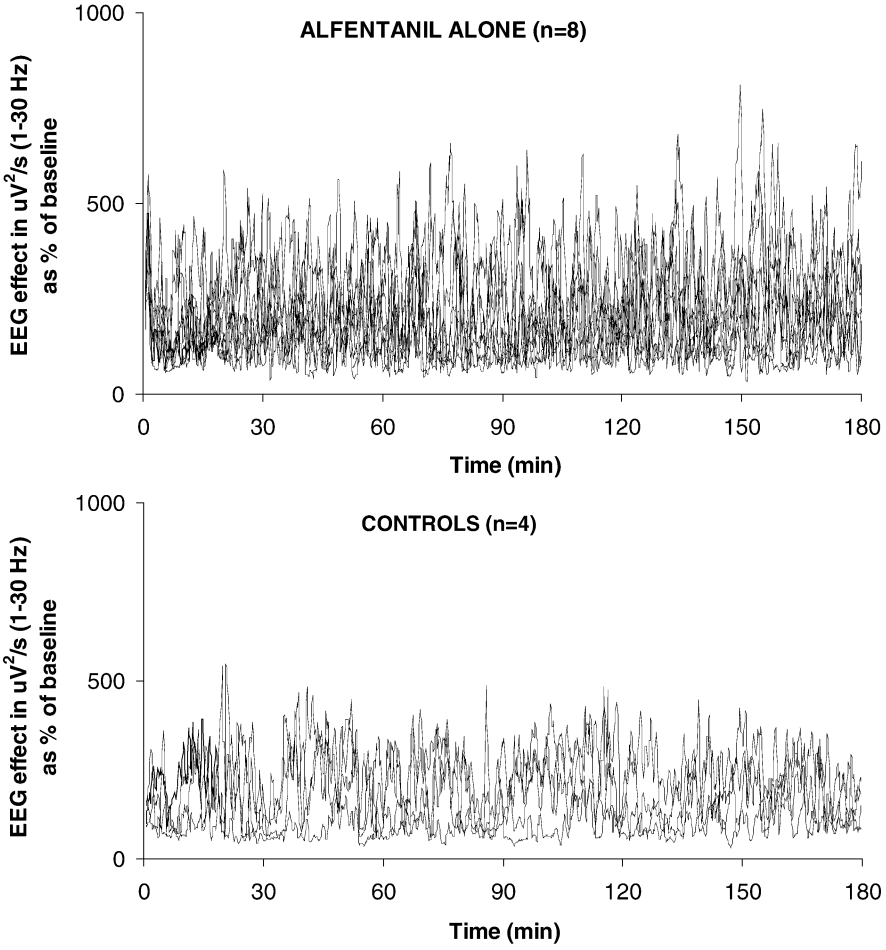

Fig. 2. Processed EEG power data ($\mu V^2/s$) from individual rats expressed as a percentage of the respective value derived from the 10-min baseline recording period in the same animal. Data are presented as the moving average over five consecutive 10-s epochs. In Group 3 (top panel), animals received alfentanil infusions commencing at 0 min delivered by a computer-controlled infusion program (Stanpump) that maintained plasma alfentanil concentrations at ~ 50 ng/ml for 180 min; in Group 4 (bottom panel), control recordings were taken from animals that received no infusion. Note the vertical scale expansion compared to Fig. 1.

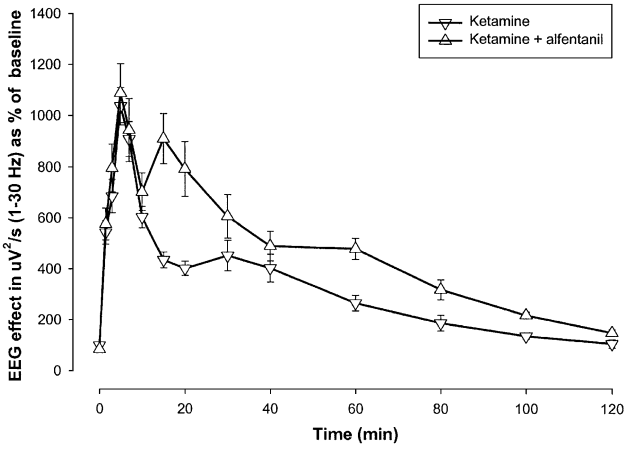

Fig. 3. Mean (and S.E.M.) values for processed EEG power data from rats at the time intervals where blood samples were taken. The medians of triplicate values for processed EEG power for the 30-s period that spanned the time intervals where blood samples were taken were expressed as a percentage of the mean processed EEG power effect ($\mu V^2/s$) from the 10-min baseline recording period. Animals in both groups 1 and 2 (each $n=8$) received ketamine infusions that commenced at 0 min and were delivered at 10 mg/kg/min for 5 min. However, in Group 2, alfentanil infusions delivered by a computer-controlled infusion program (Stanpump) commenced 7 min after ketamine infusions concluded and maintained plasma alfentanil concentrations at ~ 50 ng/ml for the remaining study period (area under the curve ketamine vs. ketamine with alfentanil: $P=0.0040$, Student's two sample t-test).

time curves were determined by the linear trapezoid method. Maximal EEG activation (E_{max}) and the plasma ketamine concentration corresponding to $E_{max}/2$ (EC_{50}) were determined by nonlinear least squares regression (SigmaPlot 4.0, SPSS, Chicago, USA) from individual sets of processed EEG power vs. plasma drug concentration data using Eq. (i) that describes a two-parameter exponential rise to a maximum. Between-group comparisons of the outcome variable data were performed by Student's two sample t-tests. Summary data are given as mean ± S.E.M.

$$y = a(1 - e^{-kx}) \quad \text{(i)}$$

where y is the value of the outcome variable and x is the corresponding plasma ketamine concentration.

3. Results

3.1. EEG effects of drugs infusions

Shortly after commencement of the ketamine infusions in group 1, the amplitude of the EEG signal increased, and intermittent slow wave-sharp wave complexes developed as previously described (Sagratella et al., 1989). By the conclusion of the infusion, the EEG signal was dominated by synchronous slow wave-sharp wave complexes which continued until approximately 10 min; thereafter, high amplitude desynchronization began to return. The EEG signal amplitude subsequently decreased progressively; scattered slow wave-sharp complexes remained until approximately 100 min, by which time the EEG signal was equivalent to baseline. By 150 min, the EEG signal contained alternate intervals of high amplitude synchronous activity and low amplitude desynchronized activity corresponding to animals changing from an inactive resting state to a state of increased arousal and alertness.

When, in group 2, alfentanil infusion (plasma alfentanil concentrations ~ 50 ng/ml) followed the ketamine infusion, the amplitude of slow wave-sharp wave complexes in the EEG signal was increased, and the EEG signal did not return to baseline until approximately 120 min. However, EEGs from animals in group 3 that received equivalent alfentanil infusions alone showed no apparent differences to those of control animals in group 4 that were not infused. In both instances, as the animal's state changed from alert to resting, the EEG alternated between low amplitude desynchronized activity and high amplitude synchronous activity.

Processed EEG power data from individual animals are shown in Fig. 1 (groups 1 and 2) and Fig. 2 (groups 3 and 4). It is clear that the infusion of alfentanil alone produced no discernible effects on processed EEG power, but marked increases in the processed EEG power followed infusions of ketamine. Furthermore, the mean area under the curve value, for the processed EEG power vs. time curve (Fig. 3), from animals in group 2 (55,335 ± 4176, $n=8$) was

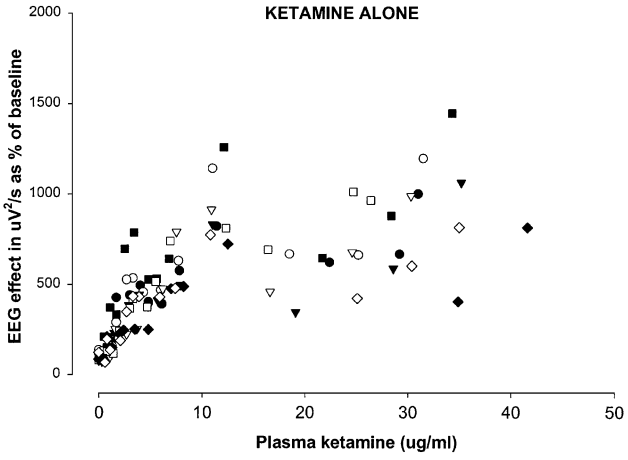

Fig. 4. Plasma ketamine concentration EEG power data from individual rats ($n=8$, shown as different symbols) following ketamine infusions that were delivered at 10 mg/kg/min for 5 min. The median of triplicate values for the processed EEG power effect ($\mu V^2/s$) over the 30 s-period that spanned the time intervals where blood samples were taken was expressed as a percentage of the mean value for the processed EEG power ($\mu V^2/s$) derived from the 10-min baseline recording period. Individual data sets for each rat were fitted to an equation describing a two-parameter exponential rise to a maximum to obtain estimates of the maximal EEG activation (E_{max}) and plasma ketamine concentrations corresponding to $E_{max}/2$ (EC_{50}).

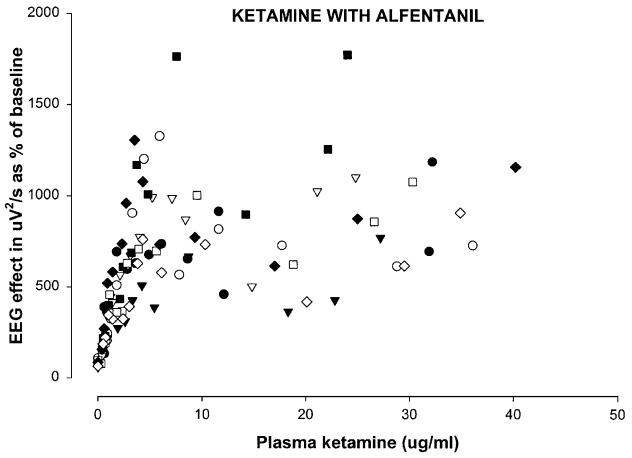

Fig. 5. Plasma ketamine concentration EEG power data from individual rats ($n=8$, shown as different symbols) following ketamine infusions that were delivered at 10 mg/kg/min for 5 min, when alfentanil infusions delivered by a computer-controlled infusion program (Stanpump) commenced 7 min after ketamine infusions concluded and maintained plasma alfentanil concentrations at ~ 50 ng/ml for the remaining study period. The median of triplicate values for the processed EEG power effect ($\mu V^2/s$) over the 30-s period that spanned the time intervals where blood samples were taken was expressed as a percentage of the respective mean value for processed EEG power ($\mu V^2/s$) derived from the 10-min baseline recording period. Individual data sets for each rat were fitted to an equation describing a two-parameter exponential rise to a maximum to obtain estimates of the maximal EEG activation (E_{max}) and plasma ketamine concentrations corresponding to $E_{max}/2$ (EC_{50}).

significantly greater (by 48%, $P=0.0040$) than that from animals in group 1 (37,439 ± 3,119, $n=8$).

3.2. Pharmacodynamic descriptors of the plasma ketamine concentration EEG power data

Plasma ketamine concentration EEG power data over 0–120 min following commencement of ketamine infusions are shown in Figs. 4 and 5. The mean R^2 values for nonlinear least squares regression of individual animal data sets in group 1 (0.78 ± 0.03, $n=7$) and group 2 (0.72 ± 0.04, $n=8$) did not differ significantly. Pharmacodynamic descriptors derived from Eq. (i) are shown in Table 1. While there was no significant difference between the mean E_{max} values for groups 1 and 2, the mean EC_{50} value from group 2 was significantly lower (by 60%, $P<0.0001$) than that for group 1. Comparison of estimates for the rate of equilibration between ketamine concentrations in plasma and the effect site (k_{e0}) as well as for effect site concentrations of ketamine (C_e), between groups 1 and 2, was precluded by the second peak in the EEG signal that followed the commencement of alfentanil infusions. This effect resulted in unrealistically low estimates of C_e in some animals, thus preventing meaningful comparison of data from a more formal pharmacokinetic–pharmacodynamic analysis.

Table 1
Mean (± S.E.M.) values for the pharmacodynamic descriptors of the plasma ketamine concentration EEG power effect relationship [maximal activation (E_{max}), and plasma ketamine concentrations corresponding to $E_{max}/2$ (EC_{50})]

Pharmacodynamic descriptors	Ketamine alone	Ketamine with alfentanil	
	Group 1 ($n=7$)	Group 2 ($n=8$)	P
E_{max} ($\mu V^2/s$)	816 ± 56	869 ± 95	0.65 (NS)
EC_{50} (μg/ml)	3.5 ± 0.3	1.4 ± 0.2	<0.0001

These were derived by nonlinear least squares regression analysis of each individual set of concentration EEG power effect data fitted to an equation describing a two-parameter exponential rise to a maximum. Animals in both groups 1 and 2 received ketamine infusions that commenced at 0 min and were delivered at 10 mg/kg/min for 5 min. However, in group 2, alfentanil infusions delivered by a computer controlled infusion program (Stanpump) commenced 7 min after ketamine infusions concluded and maintained plasma alfentanil concentrations at ~ 50 ng/ml. Between-group comparisons were performed by Student's two sample t-tests.
NS: not significant.

3.3. Behavioral effects of drug infusions

Behavioral depression and loss of righting reflex rapidly followed the commencement of ketamine infusion, although the foot withdrawal reflex to toe pinch was not lost at any point. After conclusion of the ketamine infusions, motor function began to return and the posthypnotic emergence reaction developed. Initially, animals displayed dyskinetic limb movements and head weaving, and as motor function recovered, stereotyped behavior and hyperlocomotion developed. The times to recover from ketamine anaesthesia as well as the associated posthypnotic emergence reaction are shown in Table 2, and were significantly longer in group 2 than group 1 (thermal probe removal by 130%, $P=0.00022$; sitting posture by 56%, $P=0.00054$; coordinated locomotion by 35%, $P=0.0017$; behavioral quiescence by 29%, $P=0.0002$).

Table 2
Mean (± S.E.M.) values of the behavioral end points for recovery from ketamine-induced anaesthesia and the associated posthypnotic emergence reaction

Behavioral end point	Ketamine alone	Ketamine with alfentanil	
	Group 1 ($n=7$)	Group 2 ($n=8$)	P
Thermal rectal probe removal (min)	25.0 ± 2.8	57.5 ± 5.5	0.00022
Sitting of four legs (min)	50.0 ± 4.7	77.8 ± 4.0	0.00054
Coordinated locomotor activity (min)	65.6 ± 3.8	89.1 ± 4.5	0.0017
Behavioral quiescence (min)	111 ± 5	143 ± 4	0.0002

Animals in both groups 1 and 2 received ketamine infusions that commenced at 0 min and were delivered at 10 mg/kg/min for 5 min. However, in group 2, alfentanil infusions delivered by a computer-controlled infusion program (Stanpump) commenced 7 min after ketamine infusions concluded and maintained plasma alfentanil concentrations at ~ 50 ng/ml. Between-group comparisons were performed by Student's two sample t-tests.

4. Discussion

The main finding of this study was that, compared to ketamine alone, alfentanil significantly increased both the duration of anaesthesia and the processed EEG power. The plasma ketamine concentration producing half-maximal EEG effect was significantly reduced in the presence of alfentanil. The results indicate that low plasma alfentanil concentrations potentiate the anaesthetic and EEG effects produced by ketamine which may have implications for clinical pain management.

Administration of ketamine (1–17.8 mg/kg, i.v.) to rats was previously shown to produce increases in the EEG signal amplitude and decreases in the predominant frequency, as well as to induce hyperlocomotion, stereotypy, and ataxia (Mattia et al., 1988). In this investigation, ketamine infusions produced posthypnotic amphetamine-like central stimulant effects as motor function returned during the emergence period. Ketamine-induced central stimulant effects apparently result from an increase in dopaminergic activity in the central nervous system (Dimpfel and Spuler, 1990; Irifune et al., 1991), and it has been suggested that NMDA receptor antagonists may stimulate dopamine release by disinhibition of dopaminergic systems (Imperato et al., 1990).

Others have found that ketamine (20–50 mg/kg, i.p.) dose-dependently induced increased periods of EEG desynchronization, increased signal amplitude, and caused the appearance of slow wave-sharp wave complexes which, at 50 mg/kg, were accompanied by motor excitation and impaired righting reflex (Sagratella et al., 1989). While these effects were also apparent in our investigation, the i.v. infusion of ketamine (50 mg/kg over 5 min) induced anaesthesia, and synchronous slow wave-sharp wave complexes dominated EEG signals for approximately 5 min immediately following the infusions. These effects are consistent with i.v. infusion producing higher brain ketamine concentrations than i.p injection.

Mean plasma ketamine concentrations by conclusion of infusion with ketamine were 33 ± 1 μg/ml (137 ± 6 μM) in group 1 and 32 ± 2 μg/ml (135 ± 8 μM) in group 2. From earlier work, it was reported that the brain/plasma distribution coefficient for ketamine was 6.5, and that brain ketamine concentrations of ~80–90 μg/g (336–379 μM) were associated with the induction of anaesthesia (Cohen et al., 1973). Others found rat brain ketamine concentrations of 27 μg/g (114 μM) on return of the righting reflex, after administration i.v. of 30 mg/kg ketamine (Marietta et al., 1977). It has previously been suggested that NMDA receptor antagonism only partly accounts for the anaesthetic effect of ketamine, since in the rat, a 5 mg/kg dose of ketamine given i.v. markedly attenuates the response to NMDA, and the IC_{50} for reducing NMDA-induced depolarizations in the neocortex is only 5.5 μM (Church and Lodge, 1990).

Ketamine interacts with multiple binding sites in the central nervous system that include NMDA, cholinergic, monoaminergic, and opioid receptors (Kohrs and Durieux, 1998). However, ketamine-induced anaesthesia appears to be mediated, at least in part, by NMDA receptors (Irifune et al., 1992). The ketamine enantiomers bind with highest affinity to the phencyclidine receptor in the NMDA receptor-ion channel, where the values of the respective inhibition constant (K_i) for the individual R- and S-enantiomers in guinea pig brain homogenate were reported to be 3.2 and 1.1 μM; their respective K_i values for μ-opioid receptors were found to be 28 and 11 μM (Hustveit et al., 1995). Similar differences in the S/R potency ratios of the two ketamine enantiomers have also been documented for their anaesthetic (~2-fold) and analgesic (~3-fold) effects in laboratory rodents (Marietta et al., 1977; Ryder et al., 1978).

In others studies, ketamine was reported to displace in vivo [^{3}H]-etorpine and [^{3}H]-naloxone binding to opiate receptors in brain tissue (Finck and Ngai, 1982; Smith et al., 1987). Bioassays that examined the opioid receptor effects of ketamine in guinea pig myenteric plexus, however, found that the effects of only S-ketamine were partially antagonized by naloxone, indicating that this enantiomer (predominantly) mediates opioid receptor-related effects produced by ketamine (Finck and Ngai, 1982). Thus, effects of S-ketamine mediated by μ-opioid receptors in the central nervous system may also contribute to the anaesthetic effect produced by infusions of ketamine. Interestingly, previous pharmacodynamic studies of ketamine's EEG effects found that R-ketamine appeared to act as a partial agonist relative to S-ketamine and antagonized the effect of S-ketamine (Schüttler et al., 1987). Hence, the use of S-ketamine is likely to provide additional favourable therapeutic advantages compared to ketamine in anaesthesia and pain management strategies.

Plasma alfentanil concentrations of ~50 ng/ml had no apparent effect on either behavior or the EEG signal of the animals, and the posthypnotic behavioral effects that followed infusions of ketamine in the presence of alfentanil were equivalent to those produced by infusions of ketamine alone. In the presence of alfentanil, however, the time to emerge from ketamine-induced anaesthesia was essentially doubled, indicating that alfentanil potentiated ketamine's anaesthetic effect. The other behavioral measures of recovery from ketamine-induced anaesthesia and the associated posthypnotic emergence effects were also substantially prolonged in the presence of alfentanil. In addition, alfentanil significantly increased the mean area under the curve of the processed EEG power for ketamine and significantly decreased the mean EC_{50} value for ketamine's plasma concentration EEG effect. Thus, alfentanil also acted to potentiate the EEG response to ketamine. This potentiation was maximal shortly after computer-controlled alfentanil infusions commenced, when the mean plasma ketamine concentration had decreased to 4.7 ± 0.3 μg/ml (20 ± 1 μM) and was still present at 120 min when the mean plasma ketamine concentration was only 0.45 ± 0.04 μg/ml (1.9 ± 0.2 μM).

We have recently shown that the distribution of ketamine into brain tissue was 2–3 times higher in the presence of alfentanil infusion (Edwards et al., 2002). Hence, the observed potentiation of ketamine's anaesthetic and EEG effects are likely to have resulted, at least in part, from increased uptake into and/or delayed redistribution from the CNS. In the present investigation, distribution of the individual ketamine enantiomers was not examined because the concurrent determination of the concentrations of alfentanil and ketamine in plasma samples required their analysis by gas chromatography–mass spectrometry (GC–MS) on a conventional achiral GC column. However, we have previously found evidence for the enantioselective uptake of S-ketamine into CNS tissue following washing infusions of ketamine (Edwards and Mather, 2001). It would be of particular interest to determine whether the distribution of S-ketamine into CNS tissue was increased in the presence of alfentanil infusion.

Alfentanil is considerably more selective for the μ-opioid receptor than morphine (Corbett et al., 1993), suggesting the effects of alfentanil observed in this investigation were mediated by CNS μ-opioid receptors. The EC_{50} for the plasma concentration EEG effect response to alfentanil at μ-opioid receptors was reported to be 289 ± 41 ng/ml (Cox et al., 1998a), while alfentanil concentrations of 1000–4000 ng/ml are required when alfentanil is used as a principal anaesthetic agent (Shafer and Varvel, 1991). Since these values are much greater than those used in this study protocol (Edwards et al., 2002), it is unlikely that ketamine acted to potentiate the EEG and anaesthetic effects of alfentanil.

However, it is possible that additional pharmacodynamic factors may have contributed to the potentiation, by alfentanil, of ketamine's EEG and anaesthetic effects. For instance, activation of μ-opioid receptors has been shown to enhance NMDA receptor-mediated responses (Chen and Huang, 1991). Thus, alfentanil could potentiate the effects of ketamine by facilitating the access of ketamine to PCP binding sites located in NMDA receptor-ion channels.

In the present investigation, we have shown that low plasma concentrations of alfentanil that were without EEG or behavioral effects acted to potentiate the anaesthetic and EEG effects produced by ketamine. Interactions between NMDA receptors and μ-opioid receptors have been implicated in the development of the hyperalgesia associated with injury and opioid tolerance as well as the development of tolerance to opioids (Mao et al., 1995). Low doses of ketamine (3.75 mg/kg, i.p.) were recently shown to potentiate the antinociceptive effects of fentanyl given intrathecally in the rat (Nadeson et al., 2002). Moreover, in the other experimental work, both ketamine and S-ketamine, but not R-ketamine, potentiated the antinociceptive effects of morphine following their intrathecal administration to rats (Joo et al., 2000). In view of these findings, our data would appear to indicate that low doses of opioids will potentiate the analgesic effect of ketamine following systemic administration. It is also likely that the use of S-ketamine would provide additional therapeutic advantages over ketamine in pain management strategies.

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