British Journal of Pharmacology (2000) 130, 1692–1698
www.nature.com/bjp

Ketamine potentiates cerebrocortical damage induced by the common anaesthetic agent nitrous oxide in adult rats

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1 For general anaesthesia, patients usually receive a combination of drugs, all of which are classified as γ-amino-butyric acid (GABA) agonists, with two notable exceptions – ketamine and nitrous oxide (laughing gas, N_2O) – which are antagonists of N-methyl-D-aspartate (NMDA) glutamate receptors. At clinically relevant doses both ketamine and N_2O, like other NMDA antagonists, have the potential to induce psychotomimetic reactions in humans and to cause pathomorphological changes in cerebrocortical neurons in rat brain. Because drug combinations used in clinical anaesthesia sometimes include both ketamine and N_2O, we undertook experiments to evaluate whether augmented neurotoxicity results from their combined use.

2 Ketamine and N_2O were administered alone or in combination by various dosing regimens to adult female rats for a duration of 3 h and the severity of cerebrocortical neurotoxic changes was quantified histologically. Because GABA agonists are known to protect against the psychotomimetic and neurotoxic effects of NMDA antagonists, we also evaluated whether the combined neurotoxicity of ketamine + N_2O can be prevented by certain commonly used GABA agonists.

3 When ketamine and N_2O were used in combination the neurotoxic reaction was enhanced to a degree much greater than can be explained by simple additivity. The apparent synergistic interaction was particularly striking when low doses of the agents were combined, the degree of toxic synergism at higher doses being masked by a ceiling effect. GABA agonists protected against ketamine/N_2O neurotoxicity.

4 It is recommended that this information be taken into consideration in the selection of drugs to be used in multi-agent protocols for general anaesthesia.

British Journal of Pharmacology (2000) **130,** 1692–1698

Keywords: Ketamine; nitrous oxide; laughing gas; NMDA receptor; neurodegeneration; drug interactions; anaesthesia; GABA receptor

Abbreviations: GABA, γ-amino-butyric acid; NMDA, N-methyl-D-aspartate; N_2O, nitrous oxide; PC/RSC, posterior cingulate/retrosplenial cortex

Introduction

Modern anaesthesiology relies on sophisticated monitoring techniques and the use of multiple combinations of drugs (sometimes referred to as cocktails) to render patients unconscious and insentient to pain. Drug combinations are carefully selected with an aim toward enhancing therapeutic benefits while minimizing adverse side effects. Although many drug combinations that have potentially harmful side effects have been identified and are successfully avoided, adverse reactions sometimes occur in the context of general anaesthesia for which the anaesthesiologist has no adequate explanation (Smith & Corbascio, 1986; Ghoneim & Long, 1970; Sokoll & Gergis, 1981; Edwards *et al*., 1979; Soni, 1987; Smith *et al*., 1966).

Nitrous oxide (laughing gas, N_2O) is the most commonly used inhalational anaesthetic in human medicine and dentistry. It has been widely used for over a century, during which research efforts to clarify its mechanism of action were unsuccessful until our recent observation (Jevtovic-Todorovic *et al*., 1998b; Mennerick *et al*., 1998) that N_2O blocks the NMDA (N-methyl-D-aspartate) subtype of glutamate receptor, which is a ubiquitously distributed receptor that mediates much of the excitatory neurotransmission in the mammalian brain. We found that N_2O, like other NMDA receptor antagonists, protects against neurodegeneration caused by NMDA, inhibits NMDA-induced currents in cultured hippocampal neurons (Jevtovic-Todorovic *et al*., 1998b; Mennerick *et al*., 1998), and causes a distinctive neurotoxic reaction in the rat cerebral cortex (Jevtovic-Todorovic *et al*., 1998a,b) that can be prevented by GABAergic agents (Jevtovic-Todorovic *et al*., 1998b).

It has been determined that MAC (Minimum Alveolar Concentration that prevents purposeful movements to supramaximal noxious stimulation in 50% of subjects) for N_2O is about 155 vol% for rats (Gonsowski & Eger, 1994) and 104 vol% for humans (Hornbein *et al*., 1982). However, it is not possible to administer N_2O at concentrations higher than 75 vol% under normobaric conditions without compromising oxygenation, which must be maintained at 25 vol%. Therefore, in human anaesthesia N_2O is used at concentrations up to 75 vol%. This provides only partial anaesthesia, hence the need to use other anaesthetic drugs in combination with N_2O (Hornbein *et al*., 1982). In rat experiments, in order to achieve full anaesthesia with N_2O alone, we have used a hyperbaric chamber and 2.0–2.2 atmospheres of pressure (200–220 vol%) which allows introduction of N_2O at concentrations from 120–180 vol% while maintaining the partial pressure of

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oxygen at an adequate level. We found that under these conditions, N_2O in a dose-dependent manner caused the same type of cerebrocortical neurotoxic reaction that other NMDA antagonists cause, which consists of acute vacuolar changes (extreme swelling of endoplasmic reticulum and mitochondria) in pyramidal neurons of the posterior cingulate/retrosplenial cortex (PC/RSC). We determined that the EC_{50} for producing this neurotoxic effect in rats is 117 vol% which, based on interspecies comparison of MAC values, represents about 79 vol% in humans, a concentration very close to that (75 vol%) commonly used in human anaesthesia (Jevtovic-Todorovic *et al*., 1998b).

The only NMDA antagonist other than N_2O that is used in modern anaesthesia is the well known intravenous anaesthetic, ketamine (Lodge & Anis, 1982; Franks & Lieb, 1994). Like N_2O and other NMDA antagonists, ketamine triggers an acute vacuolar neurotoxic reaction (preferentially affecting PC/RSC neurons in the adult rat brain (Olney *et al*., 1989). It has been shown that this reaction is usually transient and reversible; however, if the NMDA receptor blockade is maintained for a long period of time, the reaction progresses to irreversible neuronal degeneration (Fix *et al*., 1993). Both N_2O and ketamine are recognized as drugs of abuse that cause psychotomimetic reactions, commonly referred to as 'emergence' reactions (Reich & Silvay, 1989; Dohrn *et al*., 1993). The relationship between the psychotomimetic reaction induced by these agents in humans and the pathomorphological reaction in cerebrocortical neurons of rat brain has not been definitively clarified, but we have presented extensive evidence suggesting that disinhibition of specific neural circuits may be a common mechanism underlying these two phenomena (Olney *et al*., 1991; Olney & Farber, 1995).

The fact that N_2O and ketamine are sometimes administered in combination to human patients for anaesthetic purposes raises the important question whether these two agents interact in an additive (or synergistic) manner to increase the risk of neurotoxic consequences. In addition, the fact that GABAergic agents, which are known to prevent the neurotoxic side effects of NMDA antagonists (Jevtovic-Todorovic *et al*., 1997; 1998b; Olney *et al*., 1991; Ishimaru *et al*., 1995), are also commonly induced in anaesthesiology protocols, raises the interesting possibility that in many cases a potentially toxic combination of NMDA antagonists has been used, but the toxic consequences have been avoided (unwittingly) by inclusion of a GABAergic agent that functions as an antidote. Highly relevant to this issue is the fact that GABAergic agents are often intentionally used together with ketamine because of the clinical observation (Magbagbeola & Thomas, 1974; Fragen & Avram, 1992) that they suppress the psychotomimetic (emergence) reactions associated with ketamine anaesthesia.

In the present study we have administered N_2O and ketamine alone or in combination to adult rats to further clarify the potential of this drug combination to produce neurotoxic side effects, and have also evaluated the ability of GABAergic agents to prevent toxic side effects of this drug combination.

Methods

Animals

Sprague-Dawley adult female rats (mean weight 320 g) were used for all experiments since female rats have been shown to be moderately more sensitive than male rats to the neurotoxic effects of NMDA antagonists (Olney *et al*., 1989). In addition, there is evidence that human females are more sensitive than males to psychotomimetic effects of ketamine (Knox *et al*., 1970; Bovill *et al*., 1971). All experiments were approved by The Animal Studies Committee, Washington University School of Medicine, St. Louis, MO, U.S.A. Experiments using more than 75 vol% N_2O were conducted under hyperbaric conditions in which the N_2O/oxygen gas mixture was introduced to a pressure of 2.0–2.2 atm (200–220 vol%) (Jevtovic-Todorovic *et al*., 1998b) and sustained for the duration of a given experiment. An exposure time of 3 h was used for all experiments because we have found this duration of exposure to N_2O optimal for inducing a fully developed vacuolar reaction in PC/RSC neurons (Jevtovic-Todorovic *et al*., 1998b). As the partial pressure of N_2O was increased (from 120–180 vol%), the oxygen partial pressure was adjusted proportionately to maintain the overall pressure at 2–2.2 atm The gas mixture (N_2O/oxygen) was delivered from calibrated flowmeters with a total flow of 5 litres/min. A relief valve on the hyperbaric chamber allowed a continuous escape of gases to avoid accumulation of carbon dioxide. After initial equilibration of the N_2O/oxygen atmosphere inside the chamber, a sample of chamber gas was analysed by mass spectrometry for N_2O content by oxygen difference (Datex-type ULT-I-27-05, Helsinki, Finland). In experiments using normobaric conditions (for N_2O concentrations of 50 and 75 vol%), the same gas flow of 5 litres/min of appropriate N_2O/oxygen gas mixture was used and a relief valve was kept open, allowing for exchange of gases under constant pressure (1 atm or 100 vol%). For control purposes air under normobaric or hyperbaric pressure was substituted for the N_2O/oxygen mixture. To prevent hypothermia in our animals the hyperbaric chamber was wrapped in heating blankets that maintained the temperature inside the chamber in a range (30–32°C) that we determined was necessary to ensure normothermia, which was confirmed by measuring rectal temperature at the end of the experiment. Ketamine (from 20–80 mg kg^{-1}), pentobarbital (20 mg kg^{-1}) or diazepam (5 mg kg^{-1}) were injected intraperitoneally (i.p.) immediately before exposure to N_2O. Isoflurane (1.5 vol% inhaled) and halothane (1 vol% inhaled) were administered through a calibrated agent-specific vaporizer delivering set percentages of the inhaled anaesthetic to the chamber. For ketamine experiments, control animals were given saline injection i.p.

N_2O and oxygen tanks were purchased from Praxair Medipure; isoflurane and halothane were purchased from Ohmeda; ketamine (100 mg ml^{-1}), pentobarbital (50 mg ml^{-1}) and diazepam (5 mg ml^{-1}) were purchaed from Fort Dodge Animal Health, Abbott Laboratoriers and Elkins-Sinn, respectively, in pre-mixed ready-to-inject solution.

Histology

At the termination of the experiment, the rats were deeply anaesthetized with pentobarbital and killed by perfusion through the left cardiac ventricle with a phosphate-buffered fixative solution containing 45% paraformaldehyde and 1.5% glutaraldehyde. The brains were additionally processed for histological evaluation by methods previously described (Jevtovic-Todorovic *et al*., 1998b; Olney *et al*., 1989; 1991). The severity of the neurotoxic reaction was assessed by counting the number of vacuolated neurons present in sections cut through the PC/RS cortex at a specific rostrocaudal level (6 mm caudal to bregma), where the toxic reaction has been shown to be maximally expressed (Jevtovic-Todorovic *et al*., 1998b; Olney *et al*., 1989; 1991).

Statistical analysis

Analysis of variance (ANOVA) models were used to evaluate drug treatment effects with numbers of vacuolated PC/RSC neurons serving as the dependent variable in each analysis. Two-way ANOVAs containing two between-subjects variables, doses of ketamine and concentrations of N_2O, were used to analyse the data from experiments involving the addition of various concentrations of N_2O to fixed doses of ketamine. Following either a significant main effect of the 'fixed' drug or a significant interaction between the 'fixed' drug and the other agent, one-way ANOVAs were conducted to determine whether varying the dose of one agent at a 'fixed' dose of the other drug had a significant effect on the numbers of vacuolated PC/RSC neurons. Appropriate pairwise comparisons were conducted following significant one-way ANOVAs. In the experiment involving the administration of various inhalational or intravenous anaesthetics together with N_2O (75 vol%) plus ketamine (40 mg kg^{-1}) a one-way ANOVA was conducted to determine if vacuolated PC/RSC neuron counts differed as a function of treatment. Following the significant ANOVA, pairwise comparisons were conducted to evaluate whether the vacuolated PC/RSC neuron counts in the N_2O (75 vol%) plus ketamine (40 mg kg^{-1}) group were different from those observed in each of the other treatment groups. The Bonferroni correction procedure was used when multiple pairwise comparisons were conducted in order to maintain alpha at the 0.05 level of significance. EC_{50} levels were calculated by linear regression analysis. For analysis of the pharmacological interaction between ketamine and N_2O, a total dose fractional value was calcualted as follows: [(ED_{50} for drug 1 when combined with a fixed dose of drug 2)/(ED_{50} value for drug 1 given alone) + (ED_{50} for drug 2 when combined with a fixed dose of drug 1)/(ED_{50} value for drug 2 given alone)]. This type of analysis is referred to as an isobolographic analysis (Tallarida *et al*., 1989). Fractional values indicate what portion of the ED_{50} for a single drug administered alone is accounted for by the ED_{50} for that drug when administered in combination with another drug. Values near one indicate an additive interaction, whereas values greater than one imply an antagonistic interaction, and values less than one a synergistic interaction.

Results

In our initial experiments evaluating the dose-response to N_2O and ketamine when used individually, we found that each drug produces a neurotoxic reaction in PC/RSC that is dose dependent (Figure 1). The calculated ED_{50} for ketamine was 47.5 ± 0.35 mg kg^{-1}, and the calculated EC_{50} for N_2O was 118 ± 0.6 vol%. The PC/RSC in control rat brains (hyperbaric air or saline injection) had normal appearance and showed no signs of neuronal vacuolization.

When N_2O and ketamine were combined we found that the neurotoxic response was markedly augmented compared to the response for either drug given alone. Figure 2 illustrates the degree of neurotoxicity obtained with various doses of ketamine when administered with a fixed concentration of N_2O. If the fixed concentration of N_2O was 75 vol% (ineffective concentration), adding an ineffective dose of ketamine (20 mg kg^{-1}) resulted in a robust vacuole reaction approximately equal to the most severe reaction produced by any dose of ketamine alone. If the fixed concentration of N_2O was at its EC_{50} (approximately 120 vol%), adding various doses of ketamine resulted in a several fold greater reaction than would be expected from a given dose of ketamine by itself. The ED_{50} for ketamine when used in combination with N_2O at a fixed concentration of 120 vol% was 8.8 mg kg^{-1} which is 1/6 the ED_{50} for ketamine when given alone. Statistical analysis revealed that when a given dose of ketamine was combined with any concentration of N_2O, the number of vacuolated PC/RSC neurons was significantly greater than was produced by that dose of ketamine by itself ($P < 0.05$).

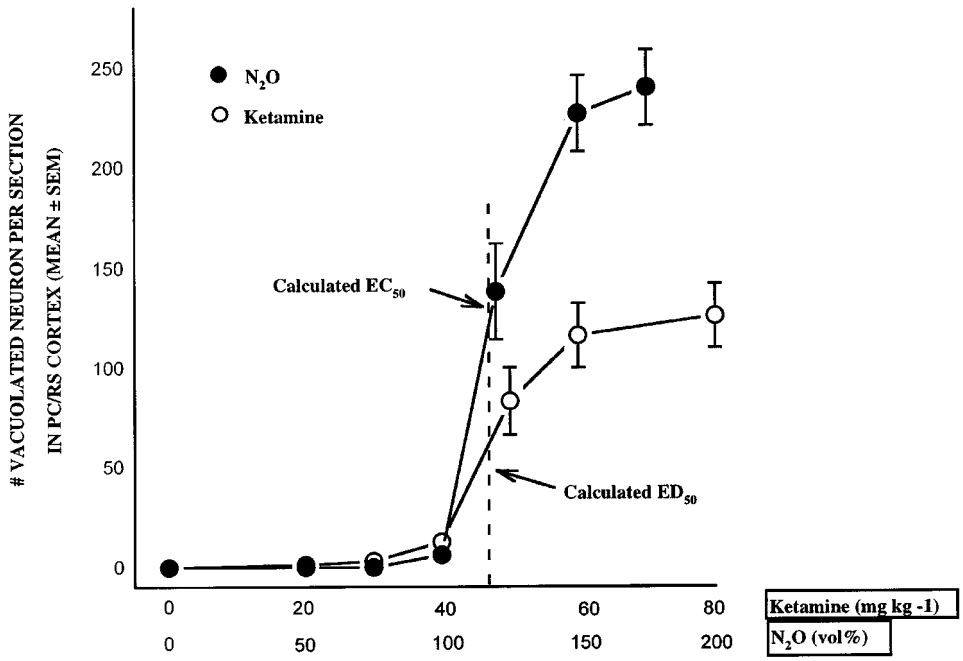

Figure 1 Neurotoxicity in PC/RSC neurons caused by exposure of adult rats for 3 h to either N_2O or ketamine is dose-dependent. Dose-dependent curves are steep and the calculated EC_{50} for N_2O is 118 vol% and calculated ED_{50} for ketamine is 47.5 mg kg^{-1} i.p. The control condition for N_2O experiments is normobaric or hyperbaric air or for ketamine experiments is saline i.p. and is depicted as 0 vol% and 0 mg kg^{-1}, respectively ($n = \geqslant 7$ per treatment group).

When various concentrations of N_2O were administered with a fixed dose of ketamine, again a marked enhancement of the neurotoxic reaction was observed (Figure 3). For example, if the fixed dose of ketamine was at its ED_{50} (approximately 40 mg kg^{-1}), adding ineffective concentrations of N_2O – 50 or 75 vol% – resulted in a 16 or 26 fold increase respectively in the toxic reaction compared to that expected from the ED_{50} ketamine dose alone. Statistical analysis revealed that when a given concentration of N_2O was combined with any dose of ketamine, the number of vacuolated PC/RSC neurons was significantly greater than was produced by that concentration of N_2O by itself ($P<0.05$). It is noteworthy that the augmentation effects depicted in Figures 2 and 3 are much more pronounced at low doses of the two agents than at higher doses, the apparent reason being that the augmentation at higher doses is masked by a ceiling effect.

Because the effect at low doses appeared to be greater than can be explained by simple additivity, we conducted an isobolographic analysis. The fractional value (the sum of the ratios of the ED_{50} values for ketamine and N_2O determined alone and jointly) was calculated to be 0.7, which supports the interpretation that the N_2O-ketamine interaction is synergistic (Figure 4).

To evaluate the potential of other anaesthetic agents, those known to act by a GABAergic mechanism, to protect against the combined N_2O/ketamine neurotoxicity, we administered various inhalational or intravenous anaesthetics together with N_2O (75 vol%) plus ketamine (40 mg kg^{-1}). As is illustrated in Figure 5, N_2O (75 vol%) (A) by itself was ineffective, and ketamine (40 mg kg^{-1}) had a very minimal effect (13 ± 3 vacuolated neurons per section in PC/RSC) (B), whereas the two agents combined had a robust effect (288 ± 17 vacuolated

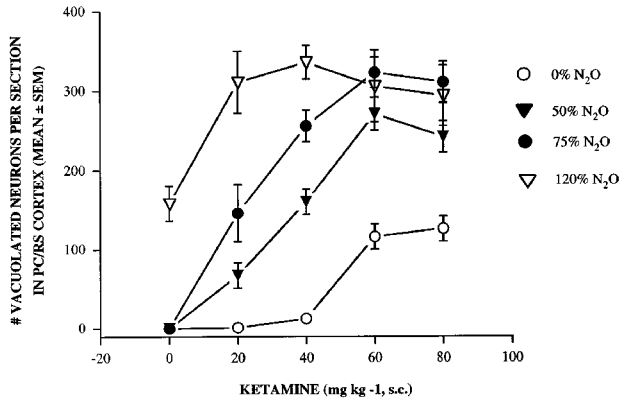

Figure 2 N_2O significantly potentiates the neurotoxicity caused by ketamine. Adult female rats were exposed to ketamine either alone or in combination with various concentrations of N_2O (as indicated) for 3 h. The ketamine neurotoxicity curve is shifted markedly upward and to the left by combination with concentrations of N_2O (50 or 75 vol%) which by themselves are not neurotoxic. The number of vacuolated neurons in PC/RSC in the presence of N_2O + ketamine is significantly higher than in the presence of the corresponding dose of ketamine alone ($P<0.05$). The control condition for N_2O experiments is normobaric of hyperbaric air or for ketamine experiments is saline i.p. and is depicted as 0 vol% and 0 mg kg^{-1}, respectively ($n=10-12$ per treatment group).

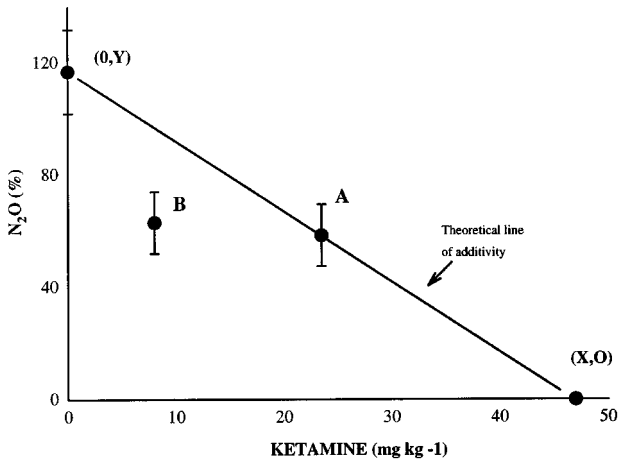

Figure 4 Isobolographic curve for the interaction between N_2O and ketamine. The EC_{50} for N_2O is plotted on the Y axis and the ED_{50} for ketamine on the X axis. The line connecting these two points is the theoretical line of additivity. Point A is the theoretical additive ED_{50} point. The experimental ED_{50} point (B) for the combination falls below the theoretical line of additivity suggesting a synergistic (supra-additive) interaction.

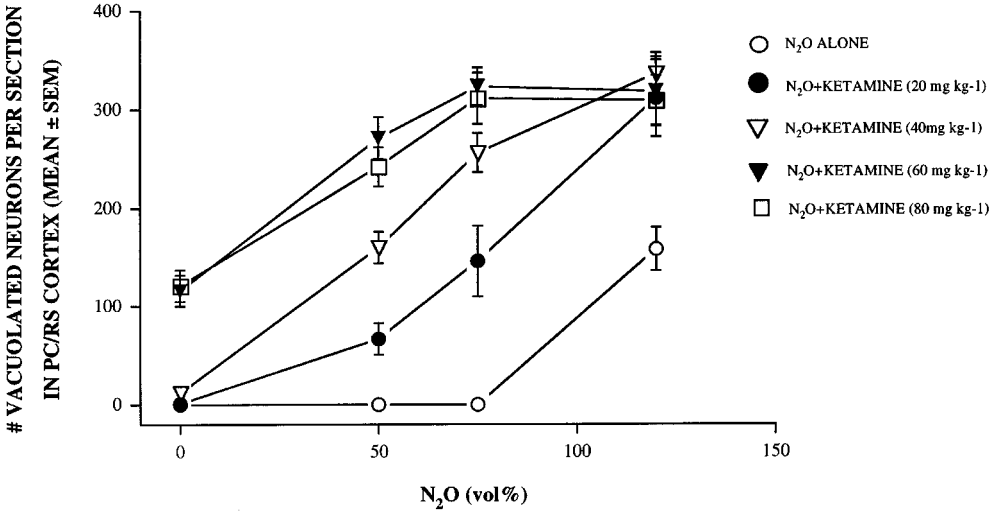

Figure 3 Ketamine significantly potentiates the neurotoxicity caused by N_2O. Adult female rats were exposed to N_2O either alone or in combination with various doses of ketamine (as indicated) for 3 h. Potentiation of N_2O neurotoxicity is particularly marked when a non-toxic concentration of N_2O is combined with a low dose of ketamine which by itself is either non-toxic (20 mg kg^{-1}) or only slightly toxic (40 mg kg^{-1}). The number of vacuolated neurons in PC/RSC in the presence of ketamine + N_2O is significantly higher than in the presence of the corresponding concentration of N_2O alone ($P<0.05$). The control condition for N_2O experiments is normobaric or hyperbaric air or for ketamine experiments is saline i.p. and is depicted as 0 vol% and 0 mg kg^{-1}, respectively ($n=10-12$ per treatment group).

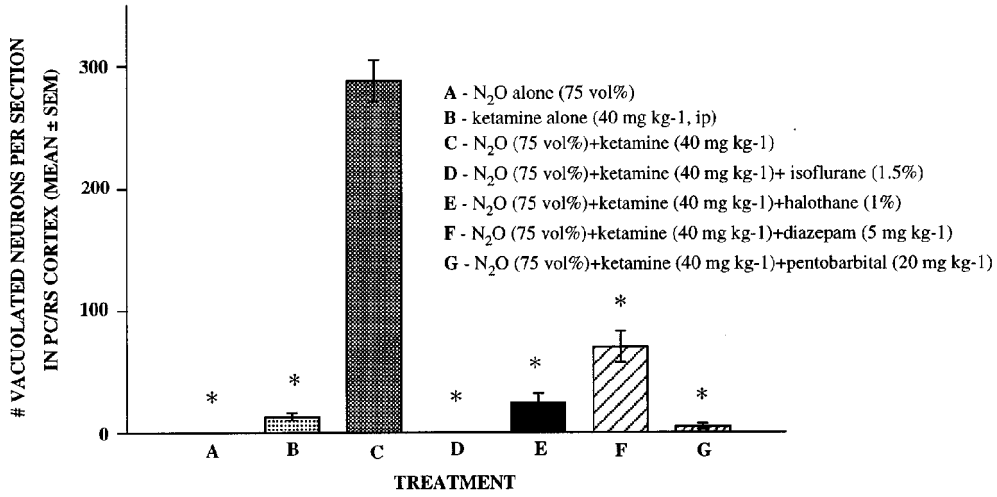

Figure 5 Either inhalational anaesthetics (halothane and isoflurane) or intravenous anaesthetics (pentobarbital and diazepam) successfully block the neurotoxic reaction caused by N_2O and ketamine. (*) = $P < 0.0005$ for comparison of D, E, F or G with C; (*) = $P < 0.0005$ for comparison of A or B with C. The P values for N_2O + ketamine as a treatment condition by itself vs N_2O + ketamine in combination with a GABAergic agent exceeded Bonferroni corrected ($P = 0.008$) levels.

neurons per section in PC/RSC) (C). The significant difference between N_2O alone or ketamine alone vs N_2O + ketamine was confirmed by one-way ANOVA and subsequent pairwise comparisons ($P < 0.0005$). In the presence of 1.5 vol% isoflurane (equivalent to 1.3 MAC for isoflurane) the N_2O/ketamine toxic reaction was completely abolished (D). Halothane, when used at 1 vol%, a concentration considered equal in anaesthetic potency to isoflurane, 1.5% vol, did not abolish but markedly diminished the N_2O/ketamine neurotoxic reaction (24 ± 8 vacuolated neurons per section in PC/RSC) (E). Diazepam 5 mg kg^{-1} strongly suppressed the reaction (70 ± 13 vacuolated neurons per section in PC/RSC) (F), and pentobarbital, at 20 mg kg^{-1} almost abolished it (5 ± 2 vacuolated PC/RSC neurons per section) (G). The significant difference between N_2O + ketamine as a treatment condition by itself vs N_2O + ketamine in combination with a GABAergic agent (isoflurane, halothane, diazepam or pentobarbital) was confirmed by one-way ANOVA and subsequent pairwise comparison ($P < 0.0005$). The P values for N_2O + ketamine as a treatment condition by itself vs N_2O + ketamine in combination with a GABAergic agent exceeded Bonferroni corrected ($P = 0.008$) levels.

Figure 6 depicts histological scenes showing the neurotoxic reaction in PC/RSC neurons caused by ketamine alone (40 mg kg^{-1}) (A), N_2O alone (75 vol%) (B), combination of N_2O and ketamine (75 vol% and 40 mg kg^{-1}, respectively) (C) and N_2O with ketamine in the presence of isoflurane (1% vol) (D). It appears that the response to N_2O/ketamine is a maximal reaction affecting all of the neurons in the PC/RSC that are potentially vulnerable, but inclusion of isoflurane in the anaesthetic protocol completely prevented the neurotoxic reaction, leaving all PC/RSC neurons looking normal.

Discussion

In this study we have demonstrated that either of two commonly used anaesthetics, N_2O and ketamine, causes an acute neurotoxic reaction in cerebrocortical neurons of the adult rat brain, and that low doses of these two agents, when combined, cause a neurotoxic reaction that is more severe than can be explained by an additive mechanism.

N_2O has long been popular in dentistry and medicine because by itself it provides significant analgesia and amnesia. However, because N_2O by itself cannot provide deeper levels of unconsciousness and analgesia required for major surgery, it is often used in combination with other more potent anaesthetics, including ketamine. Because neither N_2O nor ketamine depresses cardiorespiratory function, combined use of these agents is considered particularly appropriate for situations (e.g. trauma, elderly and obstetric patients) in which respiratory and haemodynamic depression must be avoided (Fragen & Avram, 1992; Stevens & Kingston, 1992). In fact, these agents are logical choices whenever hypotension is a potential problem because both have sympathomimetic properties that bolster blood pressure by increasing peripheral vascular resistance. Our findings show that low concentrations of N_2O and ketamine, when administered in combination, cause a severe neurotoxic reaction in the rat cerebral cortex. For example, when a non-toxic dose (20 mg kg^{-1} i.p.) of ketamine (equivalent to 3 mg kg^{-1} i.m. in humans) (Green *et al*., 1981; Fragen & Avram, 1992) was supplemented with a non-toxic concentration (50 vol%) of N_2O (equivalent to 35 vol% N_2O in humans) (Hornbein *et al*., 1982) it caused a more severe neurotoxic reaction than was produced by any dose of ketamine by itself. While interspecies comparisons do not provide a precise basis for risk evaluation, it is important to recognize that the doses of these agents used in our rat experiments, when converted into human dose equivalents, are well within the range of doses often used in human anaesthesia (ketamine is used in human anaesthesia at a dose of 5–10 mg kg^{-1} i.m., and N_2O in concentrations up to 75 vol%) (Hornbein *et al*., 1982; Fragen & Avram, 1992; Stevens & Kingston, 1992).

It has been recognized for many years that ketamine, which is a structural analogue of phencyclidine (PCP, angel dust), a well known psychotomimetic drug of abuse, induces psychotic reactions referred to as 'emergence' reactions. Because of its PCP-like properties, ketamine itself has recently become a popular drug of abuse (Knox *et al*., 1970; Bovill *et al*., 1971), known as 'Special K'. Ketamine emergence reactions include symptoms characteristic of psychosis (hallucinations and delusions) and also of delirium (agitation, confusion, disorientation and memory impairment) (Fragen & Avram, 1992; Knox *et al*., 1970; Bovill *et al*., 1971). N_2O is also known to

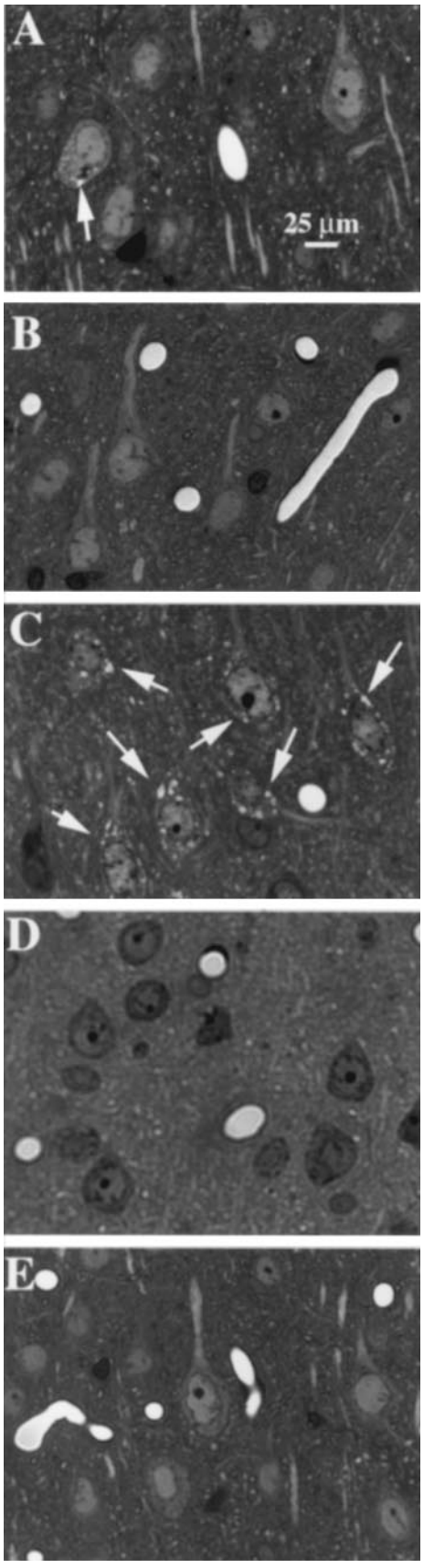

Figure 6 Histological appearance of the PC/RSC 3 h after treatment with: (A) Ketamine alone (40 mg kg^{-1} i.p.). There is a very mild toxic reaction evidenced by vacuolization of a single neuron (arrow); (B) N_2O alone (75 vol%). There is no detectable toxic reaction; (C) N_2O (75 vol%) + ketamine (40 mg·kg^{-1} i.p.). There is a severe toxic reaction evidenced by conspicuous vacuoles in all neurons in the field (multiple arrows); (D) N_2O (75 vol%) + ketamine (40 mg kg^{-1} + isoflurane (1.5 vol%). PC/RSC neurons appear normal. Isoflurane has completely blocked the neurotoxic reaction that would otherwise be caused by N_2O + ketamine . (E) Control condition – exposed only to normobaric air. PC/RSC neurons have a normal appearance (magnification all panels ×480).

cause psychotomimetic reactions, and for more than 100 years has periodically been used as drug of abuse (Dohrn *et al*., 1993). Typically, however, N_2O-induced psychotic reactions are less severe and less prolonged because N_2O, being eliminated from the body very rapidly through the lungs, has a shorter duration of action than ketamine or PCP (Fragen & Avram, 1992; Stevens & Kingston, 1992).

Although the relationship between the psychotomimetic reaction induced by these agents in humans and the pathomorphological reaction in cerebrocortical neurons of rat brain is not fully understood, a growing body of evidence (Jevtovic-Todorovic *et al*., 1998a,b; Olney *et al*., 1989; 1991) suggests that the two phenomena are mediated by a common mechanism involving disinhibition of excitatory neural pathways that heavily innervate cerebrocortical neurons. If this interpretation is correct, the symptoms of psychosis and delirium induced by anaesthetic doses of these agents (Reich & Silvay, 1989; Knox *et al*., 1970; Bovill *et al*., 1971) may represent clinically detectable psychotoxic manifestations of a process that simultaneously may be causing occult pathomorphological changes in cerebrocortical neurons. Whether such pathomorphological changes do occur in humans as a result of combined exposure to N_2O and ketamine is unknown, but it is well known that a significant percentage of patients following surgical anaesthesia manifest mental disturbances referred to variously as 'acute organic brain syndrome', 'delirium' or 'toxic psychosis'. The symptom complex observed in these patients is very similar to the symptoms of an emergence reaction. Sometimes the delirium can be traced to an identifiable cause such as fever, but often no cause is found. These unexplained adverse reactions are particularly common in the elderly and are usually but not always transient and reversible (Berggren *et al*., 1987; Lipowski, 1989). Regardless how long they last, it is difficult to trace them to any particular agent from among the many drugs that the patient, for anaesthesia or for other purposes, was exposed to perioperatively. While a toxic psychosis in a small percentage of patients is currently viewed as an acceptable risk associated with any major surgical procedure in which general anaesthesia is used, it warrants consideration that the frequency of such reactions might be reduced if the anaesthetic protocol avoids heavy use of either N_2O or ketamine and totally avoids use of the two agents together.

It has been recognized for many years that if a GABAergic agent is administered following ketamine anaesthesia, the frequency and severity of 'emergence' reactions can be reduced to a manageable level (Magbagbeola & Thomas, 1974; Fragen & Avram, 1992; Knox *et al*., 1970; Bovill *et al*., 1971). It has been shown more recently that a wide variety of GABAergic agents (benzodiazepines, barbiturates, halothane, isoflurane, propofol), if co-administered with an NMDA antagonist, are very effective in preventing the pathomorphological reaction triggered in rat brain by NMDA antagonists (Jevtovic-Todorovic *et al*., 1997; 1998b; Olney *et al*., 1991; Ishimaru *et al*., 1995). In the present study we have shown that several GABAergic agents that are commonly used in anaesthesia (diazepam, pentobarbital, halothane, isoflurane) protect against the neurotoxic reaction induced by combined treatment with N_2O and ketamine. It is noteworthy, that in clinical anaesthesia the GABAergic agent has often been given

in the postoperative recovery room, as symptoms of an emergence reaction are beginning to appear, whereas in prior research we have shown that for optimal protection against the neurotoxic reaction the GABAergic agent must be given in advance or at the same time as the NMDA antagonist drug. If the emergence reaction is, indeed, a psychotoxic manifestation of pathomorphological changes occurring in cerebrocortical neurons, applying the protective drug after the morphological changes are already taking place does not represent optimal management of this type of adverse reaction. A preventive approach in which the GABAergic protective drug is administered in advance to prevent both the neurotoxic and psychotoxic process from occurring would clearly be preferable.

In addition to the situation described above, in which a GABAergic agent has been administered intentionally to suppress emergence reactions, GABAergic drugs are often used as a component of the drug cocktail used for induction or maintenance of general anaesthesia. However, in the absence of any rational plan for introducing the GABAergic agent in a timely manner relative to when N_2O and/or ketamine are introduced, the expected outcome would be a variable and unpredictable incidence of psycho-neurotoxic reactions, which can probably be avoided by judicious application of the preventive principles discussed herein.

Supported in part by a NIDA Career FAER/ABBOTT Development Award K08 DA 00406 (V. Jevtovic-Todorovic), a New Investigator Award (V. Jevtovic-Todorovic), a NARSAD Distinguished Investigator Award (J.W. Olney) and NIH grants HD 37100, NIA AG 11355, NIDA DA 05072, NEI EY 08089 (J.W. Olney). We wish to thank J. Labruyere for technical assistance.

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(Received January 18, 2000
Revised April 17, 2000
Accepted May 12, 2000)