NITROUS OXIDE INHALATION DOES NOT INFLUENCE PLASMA CONCENTRATIONS OF β-ENDORPHIN OR MET-ENKEPHALIN-LIKE IMMUNOREACTIVITY

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Although the actual mechanism by which nitrous oxide produces analgesia remains unknown, there have been a number of reports (Berkowitz, Finck and Ngai, 1977; Chapman and Benedetti, 1979; Gillman, Kok and Lichtigfeld, 1980; Yang, Clark and Ngai, 1980) of the antagonism of nitrous oxide analgesia by the opiate receptor antagonist, naloxone. This suggests that endogenous opioid systems may be involved in the mechanism of the analgesia produced by nitrous oxide.

Recently, it was demonstrated that women in labour who received 50% nitrous oxide in oxygen as the sole analgesic agent had higher plasma concentrations of β-endorphin-like immunoreactivity (pLI) than those receiving pethidine, lumbar extradural blockade, or a combination of these (Thomas, Fletcher and Hill, 1982). These authors suggested that the administration of nitrous oxide during labour was accompanied by the release of β-endorphin (βE) into the circulation, and that higher concentrations of plasma βE following the administration of nitrous oxide might be an indication of the mechanism of its action. However, interpretation of these results is complicated by the recognized influences of stressful stimuli on plasma βE concentrations (Guillemin et al., 1977; Dubois et al., 1982).

In order to avoid such influences, we have measured the plasma concentrations of the endogenous opioid peptides βE and methionine-enkephalin (met-enkephalin, ME) in 10 normal, pain-free volunteers before and 20 min after the inhalation of a mixture of nitrous oxide in oxygen. Immunoreactive ACTH concentrations were also measured, to quantify any contribution of stress in these subjects.

**SUMMARY**

The possibility that nitrous oxide releases endogenous opioid peptides into the circulation has been tested in 10 pain-free, unstressed volunteers breathing 30% nitrous oxide in oxygen. Despite achieving plateau concentrations in venous blood, accompanied by subjective effects, there were no significant changes in plasma concentrations of immunoreactive β-endorphin, methionine-enkephalin or ACTH. These results indicate that, in the absence of nociceptive input, the effects of the inhalation of nitrous oxide are unrelated to alterations in peripheral concentrations of these endogenous opioid peptides.

**SUBJECTS AND METHODS**

Ten healthy volunteers (five male) aged 24–39 yr gave informed consent to take part in the study. Subjects attended the research laboratory on the morning of the day of investigation. An 18-s.w.g. cannula was inserted to a vein on the dorsal aspect of the forearm after infiltration with 1% lignocaine, and flushed with heparin-saline solution. The subjects then left the laboratory and continued their usual daily routine. They returned at 14.00 h and were made comfortable on a couch. Thirty millilitre of blood was sampled to measure basal concentrations of immunoreactive βE, ME and ACTH. The samples were transferred into chilled 10-ml lithium–heparin tubes and centrifuged at 2000 rev min⁻¹ at 4 °C. The tubes for the ME sample were primed with aprotinin 500 μl. After spinning for 10 min, the supernatant plasma was pipetted into chilled plastic tubes, flash-frozen in
solid carbon dioxide, then stored in a deep freeze at −70 °C. The plastic tubes for ME assay were primed with glycine hydrochloride buffer 750 μl. Measurement of plasma concentrations of βE, ME and ACTH in thawed plasma samples was performed by radioimmunoassay (Jeffcoate et al., 1978; Clement-Jones et al., 1980a; Rees et al., 1971). The between assay coefficients of variation for the βE, ME and ACTH assays were, respectively, 12%, 10% and 8%, and the sensitivities of the assays were 10 pg ml⁻¹, 5 pg ml⁻¹ and 10 pg ml⁻¹, respectively. The radioimmunoassay for βE shows equimolar cross reaction with β-lipotropin (β-LPH).

After the baseline samples had been obtained the subjects inhaled a mixture of 30% nitrous oxide in oxygen by mouthpiece from a Quantiflex dental anaesthesia machine. A nose clip was used to prevent dilution of the gas mixture with room air. Two-millilitre blood samples were taken at 5-min intervals into Hamilton gas-tight syringes containing heparin 1000 i.u. ml⁻¹, in the deadspace. Nitrous oxide concentrations were estimated on these samples by gas chromatography using a technique developed in our laboratory which gives a between-assay coefficient of variation of 1.3–2.2% (Saloojee and Cole, 1978).

After 20 min, when the blood concentration would be expected to have reached a plateau of >95% of the alveolar concentration (Eger et al., 1966; Hinds, Ellis and Saloojee, 1978), further blood samples were taken, and the nitrous oxide discontinued.

**RESULTS**

The partial pressure of nitrous oxide reached a plateau concentration of 20.9 ± 1.6 kPa (mean ± SEM) 11 min after commencing inhalation (fig. 1). All volunteers reported a subjective feeling likened to alcoholic intoxication.

In nine of the 10 subjects, basal ACTH concentrations were less than 80 pg ml⁻¹ (fig. 2). In one subject, the concentration was 87 pg ml⁻¹, and this individual also had an increased concentration of βLI (290 pg ml⁻¹; this value has been omitted from figure 3). In other individuals, basal concentrations of βLI were found to range from 14 to 124 pg ml⁻¹ (fig. 3).

**Fig. 1.** Partial pressure of nitrous oxide in venous blood (kPa) (mean ± SEM). Inhalation of 30% nitrous oxide in oxygen commenced at 0 min.

**Fig. 2.** Plasma concentrations of immunoreactive ACTH (pg ml⁻¹) before and after inhalation of 30% nitrous oxide in oxygen.

**Fig. 3.** Plasma concentrations of immunoreactive β-endorphin (pg mg⁻¹) before and after inhalation of 30% nitrous oxide in oxygen.
Immunoreactive ME concentrations were found to range from 8 to 153 pg ml\(^{-1}\) in nine subjects (fig. 4), although in one individual a value of 237 pg ml\(^{-1}\) was obtained.

There were no significant changes \((P > 0.05,\) paired \(t\) test) in plasma concentrations of immunoreactive ACTH, \(\beta E\) or ME in response to the inhalation of nitrous oxide (figs 2, 3, 4).

**DISCUSSION**

\(\beta\)-Endorphin is derived, with ACTH, from the pituitary precursor pro-opiomelanocortin (POMC) and the peptides are secreted concomitantly in stress (Guillemin et al., 1977). Foot-shock-induced stress increases the blood concentration of \(\beta E\) in rats (Rossier et al., 1977) whilst in humans, increases in the plasma concentrations of \(\beta E\) are associated with a variety of stressful situations, including hypoxia (Yanagida and Corssen, 1981), surgery (Dubois et al., 1982) and labour (Steinbrook et al., 1982; Thomas, Fletcher and Hill, 1982; Abboud et al., 1983).

The basal concentration of \(\beta LI\) measured in nine of our 10 subjects ranged from 14 to 124 pg ml\(^{-1}\). This was in keeping with the original findings of Jeffcoate and his colleagues (Jeffcoate et al., 1978), who described a range of 25–200 pg ml\(^{-1}\) in normal individuals at 09.00 h, decreasing through the day to a range of <20 – 80 pg ml\(^{-1}\) by 23.00 h. It was discovered on questioning the subject in whom the basal concentration was increased to 290 pg ml\(^{-1}\) (associated with an ACTH concentration of 87 pg ml\(^{-1}\)), that he had become involved in a rather stressful argument shortly before the basal blood samples were taken!

Immunoreactive ME circulates in human plasma (Clement-Jones et al., 1980b) and is widely distributed in the central and peripheral nervous systems (Hughes, Kosterlitz and Smith, 1977). Within the adrenal medulla, high concentrations of ME are found in association with catecholamines in chromaffin cells (Viveros et al., 1979) and, in dogs, splanchnic nerve stimulationinduces secretion of ME (Hexum et al., 1980). The plasma concentrations of met-enkephalin-like immunoreactivity (MLI) are increased in canine endotoxin shock (Evans et al., 1984) and have been described in normal women during exercise (Howlett et al., 1984), in the syndrome of chlorpropamide-alcohol flush (Medback et al., 1981) and in renal failure (Smith et al., 1981).

In nine of our subjects, the basal concentrations of MLI ranged from 8 to 153 pg ml\(^{-1}\), and accord with the original study by Clement-Jones and colleagues (1980b), who found concentrations of 14–140 pg ml\(^{-1}\) in a group of 20 normal volunteers. Our 10th subject was found to have a basal concentration of 237 pg ml\(^{-1}\). She was in all respects normal and had not exercised before blood sampling. We can offer no explanation for her high basal MLI concentration.

Antagonism of the effects of nitrous oxide by naloxone has been demonstrated in both animals (Berkowitz, Ngai and Finck, 1976; Berkowitz, Finck and Ngai, 1977) and man (Chapman and Benedetti, 1979; Gillman, Kok and Lichtigfeld, 1980; Yang, Clark and Ngai, 1980). Whilst this could indicate activation of endogenous opioid systems, it has also been suggested (Gillman and Lichtigfeld, 1983; Gillman, 1984) that nitrous oxide
may act directly as an agonist at opiate receptors. This theory is supported by evidence that prolonged exposure of rats to 80% nitrous oxide decreases opiate receptor density in the brainstem (Ngai and Finck, 1982).

If nitrous oxide were to release endogenous opioid peptides within the central nervous system, this might produce measurable changes in cerebrospinal fluid (CSF) which would not necessarily be reflected in peripheral plasma. Under normal circumstances, the blood–brain barrier is relatively impermeable to enkephalin peptides, as it is to other neurotransmitters (Cornford et al., 1978). This is borne out in obstetric patients during pregnancy and labour, in whom plasma and CSF concentrations of βE1 are dissociated (Steinbrook et al., 1982). Conversely, a correlation between plasma and CSF concentrations of βE has been demonstrated by Smith and colleagues (1982), although their study was performed in patients with severe head injury in whom the blood–brain barrier may have been disrupted. Therefore, the extent to which changes in systemic concentrations of endogenous opioid peptides reflect alterations within the central nervous system is unclear. A direct measurement of endogenous opioid activity in CSF has been obtained in patients undergoing general anaesthesia which included 70% nitrous oxide (Way et al., 1982). Although the use of other anaesthetic agents may have influenced the results of this study, no measurable increase in CSF concentration of βE was found.

Whilst any changes in the concentrations of endogenous opioids in response to the administration of nitrous oxide might conceivably be influenced by nociceptive input, this study was designed to investigate the effects of the agent in the absence of such stimuli. To this end, i.v. cannulae were inserted several hours before blood sampling began, and the experiment was performed on quiet, resting volunteers. As a result, ACTH concentrations were within the normal range in nine of the subjects. The influence of diurnal variation on peptide concentrations was avoided by sampling at the same time of day in each individual.

Under these controlled conditions, we have been unable to demonstrate any peripheral reflection of the release of BE or ME as a result of the inhalation of nitrous oxide. This supports the negative findings in CSF reported by Way and colleagues (1982). However, our results cannot exclude a direct action of nitrous oxide at opiate receptors or, indeed, the nitrous oxide-mediated release of other peptides.

REFERENCES


