ANALGESIC INTERACTION BETWEEN NITROUS OXIDE AND DELTA-9- TETRAHYDROCANNABINOL IN THE RAT

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SUMMARY
The analgesic activities of a 75:25% nitrous oxide-oxygen mixture administered for 15 min, of Δ-9-tetrahydrocannabinol (THC) 10 mg kg⁻¹ i.p., and of a combination of both, were evaluated in the rat by tail-flick and hot-plate tests. The nitrous oxide-oxygen mixture produced a significant increase in the pain threshold. The analgesic activity of THC was similar in extent but of longer duration than that of nitrous oxide. The cannabinoid also induced some locomotor and behavioural modifications. When both THC and the nitrous oxide-oxygen mixture were administered, a significant potentiation of the analgesic response was produced, without modification of the locomotor and behavioural responses that were induced by THC alone. Such mixtures may prove of value in the control of chronic pain in man.

Nitrous oxide, as a 50% oxygen mixture (Entonox), is often used to produce analgesia (Parbrook, 1968; Johnson, 1979). Δ-9-tetrahydrocannabinol (THC) also appears to be an effective analgesic (Bicher and Mechoulam, 1968; Buxbaum, 1972; Wilson and May, 1975) and might prove satisfactory for the control of pain in terminal cancer (Noyes et al., 1975; Staquet, Gantt and Machin, 1979; Poster et al., 1981) because of the absence of physical dependence and because of its antidepressant, antiemetic, cytostatic and appetite-stimulating properties (Harris, 1979; Lemberger, 1980; Kettenes-Van Den Bosch et al., 1980).

The purpose of the experiments described in this paper was to determine, in experimental animals, whether any advantageous analgesic interaction occurred between nitrous oxide and the cannabinoid, since such a combination might be of therapeutic value in the control of chronic pain.

MATERIALS AND METHODS
Male Wistar rats weighing 190–210 g were housed under constant environmental conditions and allowed access to food and water ad libitum.

The antinociceptive activities of nitrous oxide and of THC were assessed using a tail-flick latency test (D'Amour and Smith, 1941), and a hot-plate test (Woolfe and Macdonald, 1944) as partially modified by Eddy and Leimbach (1953). Both tests were carried out at the same time of day, so that any potential source of error in the activity of endogenous opiate-like substances, arising from the circadian rhythm (Frederickson, 1977), would be minimized. The experiments were carried out in a quiet room to exclude environmental interferences.

The nitrous oxide-oxygen mixture was directly and uniformly administered within each cage, in which only one animal was placed. The fenestrated Plexiglas cages were designed to facilitate the performance of the antinociceptive tests.

The THC used in the experiments was extracted with petroleum benzene from selected parts of Cannabis sativa L. After separation on a Silica gel 100 (Merck) column, using a solvent mixture of benzene, hexane and diethylamine (75:30:3), the THC obtained was tested by thin-layer chromatography and gas-liquid chromatography, and its purity was estimated at about 98%. Subsequently, the THC was dissolved in olive oil at a concentration of 10 mg ml⁻¹.

The gaseous mixture was delivered to the cages through a manifold, from calibrated flow meters, at a total flow rate of 10 litre min⁻¹.

In order to evaluate any interaction between the cannabinoid and nitrous oxide, the animals were randomly subdivided into six groups, each of 20 animals, as follows:
Group 1: exposed to 75:25% nitrous oxide-oxygen mixture for 15 min;
Group 2: i.p. administration of THC in 0.2 ml olive oil (10 mg kg⁻¹);
Group 3: i.p. administration of THC in 0.2 ml olive oil (10 mg kg\(^{-1}\)) followed immediately by exposure to 75:25% nitrous oxide–oxygen mixture for 15 min.

Group 4: exposed to a stream of air for 15 min.

Group 5: i.p. administration of olive oil (0.2 ml).

Group 6: exposed to a stream of air for 15 min immediately after i.p. administration of olive oil (0.2 ml).

The animals in each group were tested either by tail-flick test or by hot-plate test (10 animals randomly selected by each test) before treatment, and then 15, 30 and 60 min after the start of the administration of the gases, or after the injection if no gases were administered.

The results were expressed as mean ± standard deviation of the mean, and were statistically evaluated by Student's t test. Statistical significance was assumed when P values were less than 0.05.

RESULTS

The results of the tail-flick test are shown in figure 1. Essentially the same results were obtained with the hot-plate test.

The experiments demonstrated that a 75:25% nitrous oxide–oxygen mixture produced a signific-

![Fig. 1. Modifications of pain threshold, evaluated by tail-flick test. Treatment with: 75:25% nitrous oxide/oxygen mixture (group 1); i.p. administration of Δ-9-THC 10 mg kg\(^{-1}\) in olive oil (group 2); above mentioned combined drugs (group 3); and control animals as in the text (groups 4, 5, 6).]
ant increase in pain threshold at 15 min with both tests. At 30 min (15 min after the end of the exposure to the gas) the antinociceptive effect was still evident, but at 60 min the pain threshold had returned to control. No significant alterations to locomotor and behavioural responses were observed.

The analgesic activity of THC 10 mg kg\(^{-1}\) was also significant, and its effect lasted longer than that of nitrous oxide; 60 min after administration, the analgesic effect was greater than at 15 or 30 min. The THC effect was accompanied by locomotor and behavioural responses, especially a decrease in spontaneous movements, slight ataxia, restlessness, and vocalization after tactile stimulation. These effects appeared a few minutes after administration, and they persisted throughout the experiments.

When the same dose of THC was injected before the nitrous oxide-oxygen mixture, the antinociceptive response was significantly greater than the individual effects of the two substances. This potentiation was especially evident at 60 min, when the combined effect was much greater than that of THC alone, even though, at this time, the effect of the gas alone had completely disappeared. No obvious modifications of the locomotor and behavioural responses to THC were observed when the gas mixture was administered subsequently.

No modifications of the pain threshold were observed in any of the control groups (4, 5 and 6).

**DISCUSSION**

The intensity and duration of the antinociceptive effects of the nitrous oxide-oxygen mixture which were demonstrated by both of the methods used, were essentially similar to the effects described by other workers (Berkowitz, Ngai and Finck, 1977; Henriksson and Jarbe, 1971; Novelli et al., 1981). Likewise, the antinociceptive effects demonstrated with THC confirm the results obtained by others (Bicher and Mechoulam, 1968; Buxbaum, 1972; Cheser et al., 1973; Gascon and Bensemana, 1975; Wilson and May, 1975). Other workers (Henriksson and Jarbe, 1971; Stark and Dews, 1980) have also described the locomotor and behavioural effects of THC, observed here. The antinociceptive effect cannot be explained in terms of impaired locomotion, since there was no evidence of paralysis. Consequently, as others have done also, we attribute the decreased response to painful stimuli produced by THC to an action analogous to analgesia.

The analgesic action of nitrous oxide bears some resemblance to that of opiates, and may be antagonized by naloxone (Berkowitz, Ngai and Finck, 1976; Berkowitz, Finck and Ngai, 1977). The analgesic action of THC, on the other hand, is not antagonized by naloxone (Bhargava and Matwyshyn, 1980) nor by nalorphine (Cheser et al., 1973), and there is no cross-tolerance with morphine (Hirschhorn and Rosecrans, 1974; Harris, 1976). THC has been shown to modify the concentrations of some neurotransmitters (serotonin, dopamine, noradrenaline) in the brain (Gascon and Bensemana, 1975), and to exert an aspirin-like inhibition of prostaglandin synthesis (Burstein and Raz, 1972; Burstein, Levin and Varaneli, 1975).

Clearly, nitrous oxide and THC differ in the mechanisms of their analgesic effects. Our results demonstrate a pronounced synergism between the two. They provide no information with regard to the mechanism underlying the synergism, but the interaction is worthy of further study, since it may have important practical consequences in the control of chronic pain.

**REFERENCES**


INTERACTION ANALGESIQUE ENTRE PROTOXYDE D'AZOTE ET DELTA-9-2 TETRAHYDROCANNABINOL CHEZ LE RAT

RESUME
Nous avons mesuré chez le rat les activités analgésiques d'un mélange oxygène–protoxyde d'azote 25%75% administré pendant 15 min, de delta-9-tetrahydrocannabinol (THC) 10 mg kg⁻¹ i.p. et d'une association des deux par les tests du retrait de la queue et de la plaque chaufante. Le mélange oxygène–protoxyde d'azote entraînait une augmentation significative du seuil de la douleur. L'activité analgésique du THC était de même intensité mais de durée plus longue que celle du protoxyde d'azote. Le cannabinoïde provoquait également des troubles locomoteurs et du comportement. Lorsque l'on administrait à la fois le THC et le mélange protoxyde d'azote–oxygène, on entraînait une potentiatisation significative de la réponse analgésique sans modification des troubles locomoteurs ou du comportement induits par le THC seul. De tels mélanges peuvent se révéler intéressants dans le contrôle des douleurs chroniques chez l'homme.

ALANGETISCHES INTERAKTION ZWISCHEN LACHGAS UND DELTA-9-TETRAHYDROCANNABINOL BEI DER RATTE ZUSAMMENFASSUNG


INTERACCIÓN ANALGESICA ENTRE EL OXIDO NITROSO Y EL DELTA-9-TETRAHIDRO CANABINOL EN LA RATA

SUMARIO

Mediante ensayos de hornilla caliente y de latigazo de cola, se evaluaron en la rata las actividades analgésicas de una mezcla de oxígeno–oxígeno nitroso al 25:75% administrada durante 15 minutos, de 10 mg kg⁻¹ de Δ-9-tetrahidro-cannabinol (THC) y de una combinación de ambos. La mezcla de oxígeno–oxígeno nitroso hizo subir de manera significativa el umbral del dolor. La actividad analgésica del THC fue semejante en su grado pero de más larga duración que la del oxígeno nitroso. El cannabinoido también indujo algunas modificaciones locomotoras y de comportamiento. Cuando se administraron ambos productos, el THC y la mezcla oxígeno–oxígeno nitroso, se produjo una ampliación significante de la respuesta analgésica, sin modificación de las respuestas locomotoras y de comportamiento como fue el caso con el THC solo. Tales mezclas podrían comprobar su valor en el control del dolor crónico en el hombre.