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THE ANALGESIC PROPERTIES OF SUB-ANAESTHETIC DOSES OF ANAESTHETICS IN THE MOUSE

BY

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Trichlorethylene in a sub-anaesthetic concentration (0.5% v/v in oxygen) has an analgesic effect on mice, which develops slowly and reaches a maximum roughly equivalent to that produced by 5 mg/kg of methadone hydrochloride given by intraperitoneal injection. Ethyl chloride causes analgesia in sub-anaesthetic concentrations (3%) but is much less potent than trichlorethylene. Halothane (0.75%) produces slight but definite analgesia. Cyclopropane and diethyl ether have no appreciable analgesic effect. Concentrations of nitrous oxide (up to 90%) in oxygen lack any observable analgesic action on the mouse. Trichlorethylene is considerably potentiated by nitrous oxide, its anaesthetic rather than its analgesic action being affected. Nitrous oxide (40%) potentiates the analgesic rather than the anaesthetic action of halothane (0.75%). However, increasing the concentration of nitrous oxide to 60% causes the anaesthetic action of halothane to predominate.

The analgesic properties of most common inhalation anaesthetics administered in sub-anaesthetic doses have been investigated repeatedly in man (Seevers, Bennett & Pohle, 1937; Hewer & Keele, 1948; Chapman, Arrowood & Beecher, 1943; Dundee, Nicholl & Black, 1962). These results often disagree, for example Seevers *et al.* (1937), using von Frey hairs to produce pain, found that 20% nitrous oxide was ineffective, while Chapman *et al.* (1943) found that the same concentration of nitrous oxide was as effective as 15 mg of morphine sulphate. The results described here were obtained using an electrical method of testing for analgesic effects in mice. An advantage of this method is that it eliminates the subjective element always present in experiments in man, the positive response of the mouse to an electrical shock being an unequivocal squeak.

METHODS

The method and apparatus used in testing for analgesia have been described previously (Neal & Robson, 1964). Unless otherwise stated, the anaesthetic drug was administered with oxygen as the only other gas, and given concentrations are v/v.

RESULTS

Trichlorethylene. The analgesic effect of 0.5% trichlorethylene is illustrated in Fig. 1 and a summary of the actual results is given in Table 1. For comparison the analgesic effect of methadone hydrochloride (8 mg/kg, intraperitoneally) is included in Fig. 1. Trichlorethylene produced its maximum effect in about 50 min,

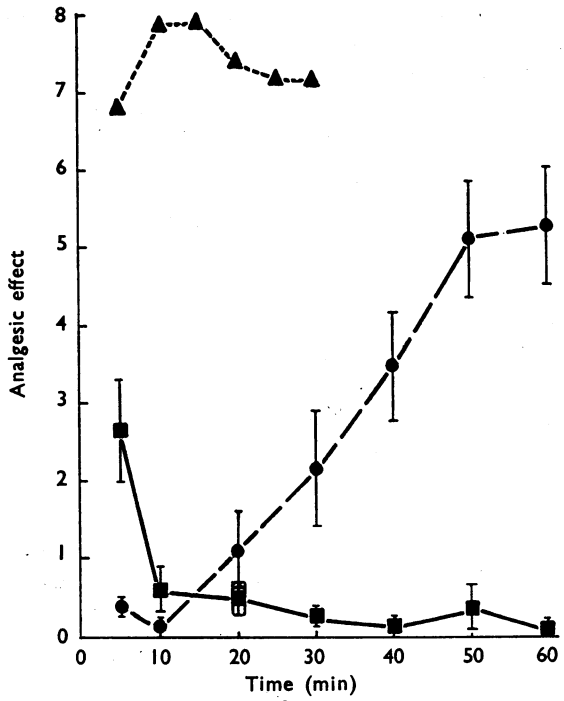

Fig. 1. The development of analgesia caused by exposing mice to 0.5% trichlorethylene in oxygen (●). The vertical lines represent the standard errors of the means. For comparison the effect of methadone hydrochloride (8 mg/kg, intraperitoneally) is included (▲). The analgesia caused by this drug comes on far more rapidly than that caused by trichlorethylene. Controls (■). The ordinate represents the number of times the mice did not squeak compared with the controls, the maximum score being 10.

TABLE 1

SUMMARY OF RESULTS OBTAINED WITH TRICHLORETHYLENE (0·5%) IN OXYGEN

The analgesic effect refers to a group of about twenty mice and indicates the number of times the mice did not squeak compared with controls, the maximum score being 10. After 30 min exposure the results differ significantly from those obtained in the control experiment using pure oxygen

Exposure time (min)	Analgesic effect (mean negative response)	Significance (*P*)
5	0·417	Not significant
10	0·125	Not significant
20	1·13	Not significant
30	2·61	<0·01
40	3·48	<0·001
50	5·12	<0·001
60	5·26	<0·001

while with methadone the maximum effect was seen in about 10 min. A concentration of 0.5% trichlorethylene was sub-anaesthetic, but increasing the concentration to 0.75% caused many mice to lose consciousness before any significant degree of analgesia occurred.

Ethyl chloride. Ethyl chloride (3%) did not produce anaesthesia in any of the mice tested. The degree of analgesia obtained with this concentration was considerably less than that obtained with 0.5% trichlorethylene. Ethyl chloride (6%) produced analgesia very quickly (Fig. 2). After exposure for 25 min the degree of analgesia was equal to the maximum obtained with trichlorethylene. However,

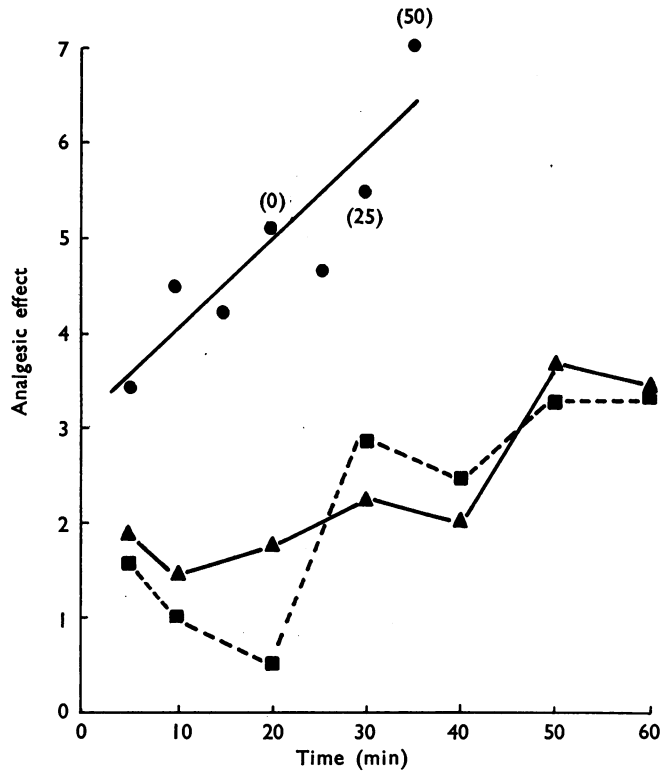

Fig. 2. The analgesic activity of ethyl chloride (3%, ■) in sub-anaesthetic doses. Ethyl chloride (6%, ●) produces a greater level of analgesia than 0.5% trichlorethylene but anaesthetizes 50% of the mice within 35 min. The mixture of 3% ethyl chloride with 60% nitrous oxide (▲) gives an action not significantly different from 3% ethyl chloride alone. Numbers in brackets indicate percentage of mice anaesthetized.

6% ethyl chloride caused loss of consciousness in 17% of the mice after this time. After 35 min exposure, 50% of the mice had become anaesthetized.

Halothane. Halothane (0.75%) had a slight but significant analgesic effect. The analgesia was slow to develop and did not become significant ($P<0.01$) until after 50 min. This concentration of halothane did not cause loss of consciousness in any mice, but increasing the concentration to 1% caused a large proportion of the mice to be anaesthetized so rapidly that no significant analgesic effect had time to develop.

Diethyl ether. Ether (3%) had virtually no analgesic effect. Only after an exposure for 1 hr was there a very slight but significant ($P<0.05$) effect. This

concentration caused loss of consciousness in some mice (17% at 50 min). Increasing the concentration of ether to 6% caused all the mice to be anaesthetized within 15 min. During this time no significant analgesia was produced.

Cyclopropane. This compound in concentrations of 5 and 10% produced no analgesia except when 10% cyclopropane was administered for 60 min, when the result was significantly different from the control ($P<0.02$). Neither of these concentrations produced loss of consciousness in any mice.

Nitrous oxide. Concentrations of nitrous oxide (up to 90%) in oxygen produced no effect on any mouse used even after 60 min. As nitrous oxide is a drug which is extensively used clinically, it was mixed with trichlorethylene and with halothane to see if the action of these drugs was influenced by the addition of nitrous oxide.

Trichlorethylene with nitrous oxide. Fig. 3 shows the effect of various concentrations of nitrous oxide on the action of trichlorethylene (0.5%). Although

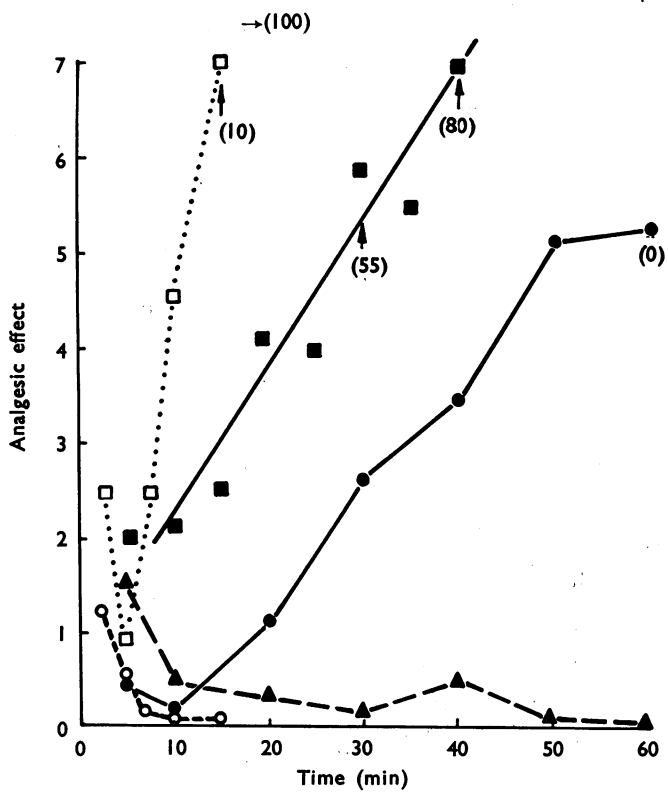

Fig. 3. The effect of various concentrations of nitrous oxide on the analgesic effect of 0.5% trichlorethylene. A mixture of 0.5% trichlorethylene and 80% nitrous oxide (□) causes anaesthesia within 20 min. The mixture of 0.5% trichlorethylene and 60% nitrous oxide (■) produces anaesthesia in 80% of mice within 40 min. Trichlorethylene 0.5% alone (●), produces no anaesthesia. ○, 0.5% trichlorethylene, 80% nitrogen and 20% oxygen; ▲, controls; numbers in brackets indicate percentage of mice anaesthetized.

nitrous oxide had no observable effect on mice when used alone it increased the action of trichlorethylene. It was mainly the anaesthetic rather than the analgesic action of trichlorethylene that was affected.

The maximum analgesic effect obtained with 0.5% trichlorethylene was the same when either 60% or 80% nitrous oxide was used. The maximum effect of these mixtures appeared to be approximately 25% greater than the maximum analgesic effect produced by 0.5% trichlorethylene alone, but only in the case of 80% nitrous oxide was the increase in analgesic effect statistically significant. Although the degree of analgesia was not greatly increased the time of onset was markedly reduced. Thus, while 1 hr was required to reach a maximum analgesic effect with 0.5% trichlorethylene alone, only 11 min were required to produce the same degree of analgesia when trichlorethylene was given with 80% nitrous oxide.

Although the analgesic effect was only slightly increased, the anaesthetic property of trichlorethylene was greatly potentiated. Thus, while 0.5% trichlorethylene in oxygen caused no mice to be anaesthetized within 1 hr, the addition of 60% nitrous oxide caused 80% of the mice to lose consciousness within 35 min and some of the mice were anaesthetized within 20 min. The addition of 80% nitrous oxide

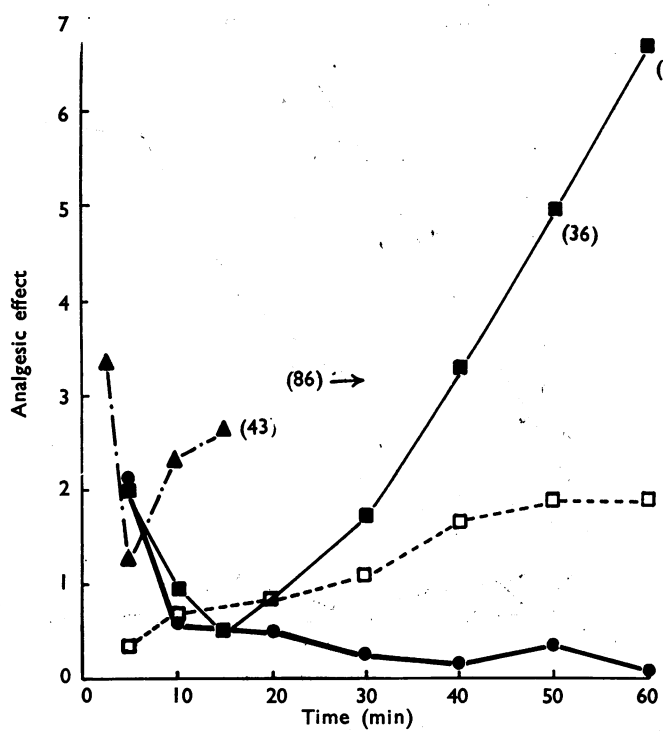

Fig. 4. The poor analgesic action of halothane 0.75% in oxygen (□). The mixture of this concentration of halothane with 40% nitrous oxide (■) causes a great increase in analgesic action and 45% of the mice are anaesthetized after 1 hr. 0.75% halothane and 60% nitrous oxide (▲) causes anaesthesia in 86% of the mice within 20 min without the development of any significant analgesia. ●, controls. Numbers in brackets indicate percentage of mice anaesthetized.

to 0.5% trichlorethylene further increased the anaesthetic effect, and all the mice became anaesthetized within 20 min. That the effect was due to the nitrous oxide, and not merely to the reduction in oxygen concentration, was shown by a control experiment in which the nitrous oxide was replaced by nitrogen.

Halothane with nitrous oxide. The potentiating effect of nitrous oxide on the action of halothane (0.75%) is shown in Fig. 4. Nitrous oxide (60%) markedly potentiated the anaesthetic action of 0.75% halothane, 86% of the mice being anaesthetized within 20 min. This combination of drugs showed little analgesic action. However, reducing the concentration of nitrous oxide to 40% produced a mixture in which the analgesic action of halothane was greatly potentiated. Although the anaesthetic effect was also increased this was less marked and, after 1 hr, less than 50% of the mice were anaesthetized. The degree of analgesia obtained with this mixture after an exposure for 1 hr was significantly greater than that obtained with halothane alone ($P<0.001$).

DISCUSSION

In man it has been shown that all the commonly used anaesthetics, with the exception of halothane, produce analgesia when administered in sub-anaesthetic doses (Dundee *et al.*, 1962). These results have not been obtained in mice. In contrast to its action in man, sub-anaesthetic doses of halothane (0.75%) produce a slight but significant degree of analgesia in mice. On the other hand cyclopropane (4 to 5%), which causes analgesia in man (Dundee *et al.*, 1962; Seevers *et al.*, 1937), has only a very slight action in mice, even when the concentration is increased to 10%. Similarly sub-anaesthetic concentrations of diethyl ether have only a very weak analgesic action in mice, although in man ether in concentrations of 1 to 2% seems to be almost as effective as 25% nitrous oxide (Dundee *et al.*, 1962). In the case of ethyl chloride no reference has been found to any analgesic properties it may have in man. In mice the degree of analgesia produced by 3% ethyl chloride is second only to that produced by 0.5% trichlorethylene. The clinical use of ethyl chloride as an analgesic agent is precluded by the frequency with which it is followed by headache, nausea and vomiting. It also depresses uterine tone in labour and is therefore quite unsuitable for use in obstetrics.

It is interesting that in the mouse 0.5% trichlorethylene produces a higher degree of analgesia than any other single agent. An important difference in the analgesic action of trichlorethylene in man and mice is the time of onset of the analgesia. In man, an analgesic effect occurs after a few minutes (Wylie & Churchill-Davidson, 1962), while in mice 30 min are required before a significant analgesic effect is evident. It is surprising that 90% nitrous oxide has no effect on mice, even after exposure for 1 hr. This contrasts markedly with the result obtained in man. Chapman *et al.* (1943) found that the concentrations of nitrous oxide required to produce loss of consciouness in seven men were, respectively, 60, 60, 65, 75, 50, 66 and 50% giving an average value of 61%. Similarly Grey (1954) showed that inhalation of 50% nitrous oxide causes loss of consciousness, but it should be noted that his results were obtained from only twelve volunteers. However, although nitrous oxide does not have any anaesthetic or analgesic effect in mice when used

alone, it does potentiate the action of other anaesthetic drugs. The addition of 80% nitrous oxide to 0.5% trichlorethylene results in a mixture which anaesthetized all the mice within 20 min. The analgesic effect was not greatly enhanced although the increase was significant in the case of 80% nitrous oxide. The same potentiation of anaesthetic properties as opposed to analgesic properties also occurs with lower concentrations of nitrous oxide when the analgesic action was not significantly increased. From the results obtained it is clear that while nitrous oxide may increase slightly the analgesic action of trichlorethylene its most marked effect is the potentiation of the anaesthetic action of trichlorethylene.

The action of nitrous oxide on the affects of halothane (0.75%) is interesting in that a differential effect can be obtained by using different concentrations of nitrous oxide. Nitrous oxide (60%) markedly potentiates the anaesthetic action of halothane without influencing its analgesic properties, but by lowering the concentration of nitrous oxide to 40% the analgesic action of halothane is greatly increased while the anaesthetic action is only moderately increased. Obviously these results cannot be applied directly to man. To the best of our knowledge no-one has ever tried subanaesthetic combinations of anaesthetics in man with a view to producing analgesia. Work is being carried out at present to ascertain whether nitrous oxide potentiates the analgesic action of trichlorethylene or halothane in man. It may be that a suitable mixture of nitrous oxide with trichlorethylene or halothane would be more effective in producing analgesia than the present methods in everyday clinical use.

The mechanism by which nitrous oxide potentiates the effects of other anaesthetics is not clear. General anaesthetic drugs are thought to produce their effects by interfering with synaptic transmission (Wyke, 1960), particularly in the multisynaptic systems forming the reticular formation and the cortical mantle. On the other hand, the classical long-tract sensory pathways and their thalamocortical relays involve relatively few synapses. Thus these systems are very little affected by general anaesthetic drugs, even in deep surgical anaesthesia. King (1956) showed this differential effect by direct recording from the reticular and thalamic relay systems in cats. The simplest way of accounting for the lack of effect of nitrous oxide in mice is to assume that the reticular and cortical projection systems in mice do not involve as many synapses as in man. Thus although nitrous oxide may influence synaptic transmission in the mouse, the interference is not sufficient to produce anaesthesia or analgesia. However, when given with trichlorethylene, the two drugs have an additive effect and this is sufficient to produce anaesthesia.

The mechanism by which sub-anaesthetic doses of anaesthetics produce analgesia is unknown. Barbiturate anaesthetics have a marked effect on the reticular system but have been repeatedly shown to be devoid of analgesic activity (Hart & Weaver, 1948 ; Dundee, 1960). It can only be assumed that, whatever the mechanism of analgesia may be, it is not due to a general deactivation of the brain by a reduction in activity of the reticular system.

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