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Convulsions in Mice after Anesthesia

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The authors previously had found that mice exposed to an anesthetizing concentration of nitrous oxide (1.45 atm) for only one to two hours would convulse when picked up by the tail following anesthesia.[1] In the present study they examined seven other anesthetics to discover whether this withdrawal (dependence) phenomenon was unique to nitrous oxide. Groups of mice were exposed to an ED_{50} of each agent for different periods of time and were tested for the incidence of withdrawal convulsions until they disappeared. Withdrawal convulsions occurred after 4–15 min of exposure to nitrous oxide, ethylene, and cyclopropane and (to a very slight extent), diethyl ether, and disappeared within 90 min in every instance. Convulsions were not elicited following exposure to halothane, enflurane, isoflurane, or fluroxene. The withdrawal syndrome following exposure to nitrous oxide was not changed by pretreatment with either α-methyldopa or reserpine, and thus a possible relationship between sympathomimetic action of the agents and the production of dependence appears unlikely. (Key words: Anesthetics, gases: cyclopropane; ethylene; nitrous oxide. Anesthetics, volatile: diethyl ether; enflurane; fluroxene, halothane; isoflurane. Brain: convulsions. Potency: dependence; ED_{50}; tolerance.)

WHEN a chronically administered depressant drug is discontinued, a withdrawal syndrome characterized by hyperactivity and sometimes convulsions may result. We previously observed that mice manifested such a syndrome after an acute (1–2-hour) exposure to nitrous oxide at 1.45 atm, a concentration that abolished the righting reflex in 50 per cent of the animals (ED_{50}).[1] The withdrawal syndrome, elicited by lifting the mouse gently by the tip of the tail, was characterized by grimacing, violent jerking and twirling, forward-pointing ears, and crossed forepaws.[2] Since this finding has implications relevant to excitement or delirium during recovery from anesthesia, we wished to determine whether this phenomenon was unique to nitrous oxide or whether it was produced by other inhaled anesthetics.

Methods

We exposed separate groups of 12 unrestrained male ICR mice for 15 and 60 min in a pressure chamber to approximately the ED_{50}s of nitrous oxide, ethylene, cyclopropane, halothane, enflurane, isoflurane, fluroxene, and diethyl ether. The pressure chamber was equipped with internal and external water-circulating temperature-control coils. Chamber gases were mixed and pulled through carbon dioxide absorbant by a fan. The mice were housed together in a 7 × 12 × 5 inch-deep polycarbonate cage with a wire top and no bedding material. Rectal probes were inserted in two additional restrained mice and chamber temperature adjusted to maintain rectal temperatures between 36.5 and 38 C. After the chamber had been flushed with oxygen for 10 min, nitrous oxide or ethylene was admitted to the chamber to the desired partial pressure and the clock started. The cyclopropane partial pressure was increased without increasing the total pressure by releasing a known reproducible volume of cyclopropane from two 1.5-l calibrated "jumbo" syringes into the chamber with the outlet valve open. The anesthetic concentration in the chamber was checked by gas chromatography, which showed that concentrations were reproducible to ±3 per cent of the desired values. Each volatile agent was vaporized in a conventional vaporizer at a high flow rate; the total flow passed through the chamber. We loaded the chamber by using initial concentrations two to three times the desired concentrations. The chamber effluent concentrations were monitored by gas chromatography and observed to increase in the usual inverse exponential manner. Rapid mixing inside the chamber ensured that the mice were not exposed to anesthetic concentrations greater than those desired. The inflow concentration was decreased to the appropriate level and the clock started when the chamber effluent reached the desired concentration (usually 6–7 min after commencing).

All mice were tested for the above-described stimulus-elicited convulsions before exposure. No mouse convulsed before exposure. At the end of the exposure period the chamber was decompressed whenever necessary, and the mice were tested for the withdrawal syndrome by two observers (testing six mice each) 1 min after removal from the chamber. Both observers knew which agent and concentration were being tested. Thereafter all the mice in a group were tested every 15 min until no convulsions occurred in two consecutive tests.

In addition to the 15- and 60-min exposure periods, separate groups of 12 mice each were exposed to ED_{50} concentrations of nitrous oxide, ethylene, and cyclo-

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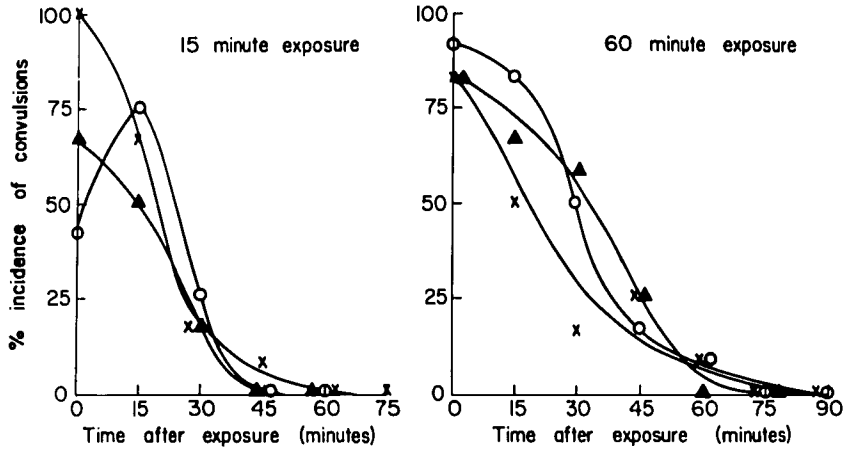

FIG. 1. The incidences of stimulus-elicited withdrawal convulsions in mice after 15 min of exposure (*A*, *left*) or 60 min of exposure (*B*, *right*) to nitrous oxide (○), ethylene (×) and cyclopropane (▲) decay to zero in 60 to 75 min.

TABLE 1. Percentage Incidences of Stimulus-Elicited Convulsions in Mice after Exposures to Anesthetic Agents*

	Exposure (Min)	Concentration: Per Cent atm	Concentration: ED_{50}	n	Percentage Showing Convulsions at a Given Time after Exposure: 1 Min	15 Min	30 Min	45 Min	60 Min	75 Min	90 Min	105 Min
Nitrous oxide	2	153	1.06	12	0	0						
	4			12	42	25	8	8	0	0		
	8			12	58	42	0	0				
	15			12	42	75	25	0	0			
	30			12	25	83	42	33	8	8	0	0
	60			12	92	83	50	17	8	0	0	
Ethylene	2	124	0.95	12	50	0	0					
	4			12	58	25	8	8	0	0		
	8			12	92	75	17	0	0			
	15			12	100	67	17	8	0	0		
	30			12	83	58	33	0	0			
	60			12	83	50	17	25	8	0	0	
Cyclo-propane	2	13.4	1.03	12	33	0	0					
	4			12	50	17	0	0				
	8			12	92	50	17	0	0			
	15			12	67	50	17	0	0			
	30			12	58	50	58	25	8	0	0	
	60			12	83	67	58	25	0	0		
Halothane	15	.710	1.30	12	0	0	0	0	0			
	60			12	0	0	0	0	0	0		
Enflurane	15	1.36	1.30	12	0	0	0	0				
	60			12	0	0	0	0	0			
Isoflurane	15	.638	1.13	12	0	0	0	0	0	0		
	60			12	0	0	0	0	0	0		
Fluroxene	15	3.39	0.98	12	8	0	0	0	0			
	60			12	0	0	0	0	0			
Diethyl ether	8	3.57	1.12	24	0	0	0	0				
	15			24	33	4	0	4	0	0		
	30			9	33	0	0					
	60			12	0	0	0	0				
N_2O + ip saline solution	30	158	1.09	12	58	58	25	8	0	0		
N_2O + ip α-methyldopa	30	158	1.09	12	50	50	17	0	0			
N_2O + ip reserpine	30	158	1.09	7	86	71	57	29	29	29	14	0

* The incidence of convulsions before exposure was always zero.

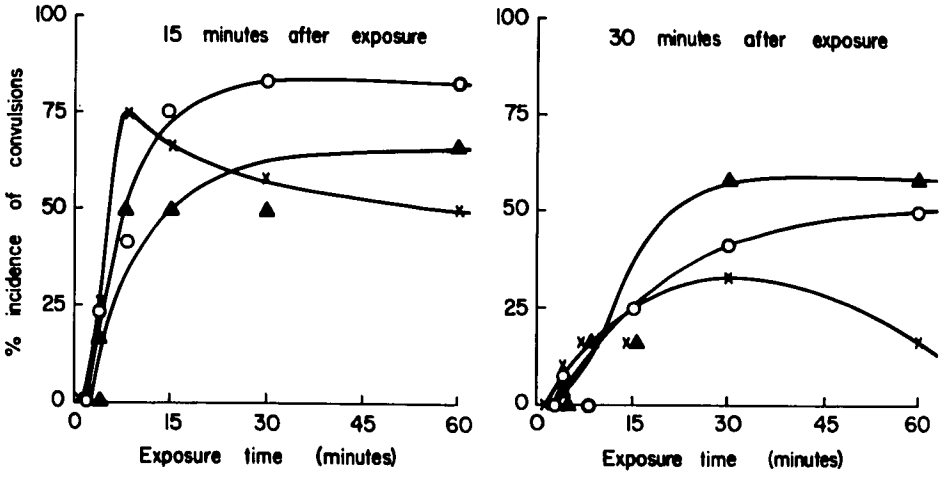

FIG. 2. The incidences of convulsions following exposure to nitrous oxide (○), ethylene (×) and cyclopropane (▲) increase with increasing durations of exposure to maxima after 15–30 min of exposure. The graphs depict the data obtained 15 (*A*, *left*) and 30 (*B*, *right*) min *after* exposure.

propane for periods of 2, 4, 8, and 30 min. Exposures to diethyl ether at its ED_{50} were made for 8 (24 mice), 15, and 30 min, in addition to the original 15- and 60-min exposures.

In a final experiment, three groups of 12 mice each were pretreated with saline solution (control), α-methyldopa (400 mg/kg), or reserpine (2 mg/kg), injected ip. The injections were repeated 24 and 36 hours later, together with 1 ml/mouse of dextrose, 5 per cent, injected sc. These concentrations of α-methyldopa and reserpine decrease halothane MAC in rats by about 31 per cent.[3] Twelve hours after the last treatment, the mice were tested for the incidence of convulsions before exposure to an ED_{50} concentration of nitrous oxide for 30 min. Following exposure these mice were tested in the manner described above.

Results

The gaseous agents, nitrous oxide, ethylene, and cyclopropane, all produced similar incidences of withdrawal and similar rates of disappearance of the withdrawal syndrome for equal periods of anesthetic exposure (table 1; fig. 1). While the results varied considerably, in general, longer exposures resulted in slower disappearance of the withdrawal syndrome. Exposure for 30 min appeared to produce maximal dependence, although an 8-min exposure to ethylene caused the highest incidence of convulsions 15 min after exposure (fig. 2). No one of these agents appeared to produce greater dependence than the others.

Of the volatile agents studied, only diethyl ether caused withdrawal after 15- or 30-min exposures, and three mice died during the 30-min exposure. The incidence with diethyl either was lower than those seen with nitrous oxide, ethylene, or cyclopropane. Although the fluroxene-treated animals appeared more excited than those exposed to halothane, enflurane or isoflurane, they did not have convulsions.

Pretreatment with α-methyldopa did not protect against the development of dependence after a 30-min exposure to 1.58 atm nitrous oxide. Reserpine similarly failed to abolish the withdrawal syndrome, but five of these mice died during exposure to nitrous oxide. The remaining mice appeared to be more susceptible to development of withdrawal syndrome than the mice given saline solution.

Discussion

Our data suggest that acute dependence occurs with some, but not all, inhaled anesthetics. We have shown that dependence, characterized by convulsions elicited by suspension by the tail, develops in mice following their exposure to ED_{50} of concentrations of nitrous oxide, ethylene, cyclopropane, and diethyl ether. The incidences of dependence seen with nitrous oxide, ethylene, and cyclopropane increase with the duration of exposure to 30 min, and the maximum incidence of convulsions was 70 per cent. The dependence we observed with diethyl ether was apparently unrelated to exposure time.

One possible explanation why some anesthetic agents do not produce dependence is that their rates of uptake and elimination may be too slow. The continuing presence of the agent may suppress the appearance of dependence. This might explain why nitrous oxide, ethylene, and cyclopropane, which all reach equilibrium very quickly, produce dependence, while halothane, enflurane, fluroxene, and isoflurane do not. However, diethyl ether, which reaches equilibrium most slowly of all the agents studied,[4] did produce dependence. Thus, the rate of uptake would not seem to be of primary importance. Further-

more, we studied mice with each of the volatile agents for at least an hour following exposure in most experiments in case the withdrawal should appear later as the agent was eliminated. No evidence of withdrawal was seen.

We thought that the withdrawal syndrome might be the result of sympathomimetic activity of the agent. However, nitrous oxide, cyclopropane, ethylene, diethyl ether, fluroxene, and isoflurane have all been reported to produce sympathetic nervous system stimulation, while halothane and enflurane do not,[5,6] suggesting that sympathetic stimulation is not important. Moreover, reserpine, which depletes central nervous system stores of catecholamines and 5-hydroxytryptamine, and α-methyldopa, which depletes norepinephrine and dopamine stores,[7] failed to abolish the development of the withdrawal syndrome. It would appear, therefore, that dependence is not a function of the sympathomimetic properties of the anesthetic agents.

We have previously demonstrated rapidly developing acute tolerance to nitrous oxide and ethylene in the presence of 13.6 atm helium in mice.[8] The results of the present study suggest that at least for these two gases, tolerance and dependence develop concomitantly, and may have a common biochemical or neurophysiologic mechanism. Other central nervous system depressant drugs, such as barbiturates and ethanol,[9,10] also produce parallel development of tolerance and dependence. If the two phenomena do develop in parallel, then rapidly developing acute tolerance should occur with cyclopropane and diethyl ether. We previously failed to find such tolerance for cyclopropane, but ascribed that failure to the slower uptake of that agent.[8]

Our results may have some clinical implications. Do the stimulus-elicited withdrawal convulsions we have found in mice have any parallel in man during recovery from anesthesia—*i.e.*, are they indicative of a propensity to produce delirium or excitement? If there is a parallel, then measurements such as ours might be used to assess the relative potentials of different anesthetic regimens to reduce these postanesthetic problems.

Halothane (Fluothane®) for these experiments was donated by Ayerst Laboratories, while enflurane (Ethrane®), isoflurane (Forane®), and fluroxene (Fluoromar®), were given by Ohio Medical Products Division of Airco, Inc.

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