Anesthesiology
V 45, No 4, Oct 1976

Amnesic Actions of Diazepam and Scopolamine in Man

M. Jack Frumin, M.D.,* Vilas R. Herekar, M.D.,† Murray E. Jarvik, M.D., Ph.D.‡

In man, diazepam alone and in combination with scopolamine interferes with the memory of visual and painful stimuli. With a 15-minute interval between injection of the drug and the showing of emotionally neutral pictures, scopolamine (0.5 mg/70 kg) produces 14 per cent forgetting when evaluated 24 hours later. Under these conditions diazepam (10 mg/70 kg) produces 41 per cent forgetting, while the combination causes 64 per cent. Under conditions designed to insure selection of subjects in whom registration was clearly quite intact at the time of the initial exposure to the pictures, memory was still found to be impaired when tested 24 hours later. Graded doses of diazepam to as much as mg/70 kg in combination with 0.5 mg/70 kg scopolamine produced a virtually linear dose–response curve for amnesia. These results are compatible with the interpretation that the diazepam–scopolamine mixture interferes with memory by blocking consolidation of the memory trace. (Key words: Parasympathetic nervous system: scopolamine: Hypnotics, benzodiazepines, diazepam: Memory, scopolamine, diazepam.)

SOME DRUGS used for preanesthetic sedation impair memory for events perceived while under their influence. This effect has been recognized since the turn of the century, when the production of "Dämmersachlaf," or twilight sleep, or morphine and scopolamine in obstetrics was reported.[1] Scopolamine has been used extensively in experimental animals for producing memory loss,[2–4] but there are relatively few quantitative studies of this action in man.[5–7]

Diazepam (Valium), an antianxiety drug, is used in man for preanesthetic medication, and has been reported incidentally to produce some loss of memory.[8,9] It has, therefore, been used before cardioversion.[10] Dundee *et al.* evaluated quantitatively the anterograde amnesia following both intramuscularly and intravenously administered diazepam given alone or in combination with meperidine and scopolamine.[11–13] Similarly, Grove-White and Kelman showed impairment of short-term memory with a relatively small dose of diazepam.[14] The literature on diazepam amnesia has been reviewed recently.[15] We decided to quantify with greater precision and detail in man the amnesic actions of scopolamine and diazepam regarding both visual and painful stimuli, possibly thereby gaining insight into mechanisms of memory impairment associated with preanesthetic drugs in terms of current psychological theory.

Memory may be considered to occur in three successive stages: registration, retention and retrieval. Storage of learned information is thought to include a consolidation process, which is inferred from the increasing resistance to disruption of the memory trace as a function of time. Amnesia is an impairment of memory caused by an interaction with any or all of these stages. Many drugs can block memory by interfering with registration if the dose is high enough, but a few drugs, notably centrally acting anticholinergics, have been shown to impair selectively retention (or consolidation) in subjects who are not sedated.[16] Barondes has discussed the relationship between amnesia and sedation.[17]

* Associate Professor of Anesthesiology. Presently Clinical Professor of Anesthesia, Stanford University School of Medicine, Stanford, California 94305.

† Instructor of Anesthesiology.

‡ Professor of Psychiatry and Pharmacology. Presently Professor of Psychiatry and Pharmacology, University of California, 760 Westwood Plaza, Los Angeles, California 90024; Chief, Psychopharmacology Research Unit, Veterans Administration Hospital, Brentwood, Los Angeles, California 90073.

Received from the Departments of Anesthesiology, Pharmacology and Psychiatry, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York 10461. Accepted for publication April 12, 1976. Supported in part by grants from the National Institutes of Health, NIH 05319 and NIH 01225, and from Roche Laboratories. Presented in part at the annual meeting of the American Society of Anesthesiologists, San Francisco, California, October 1969.

Address reprint requests to Dr. Jarvik.

TABLE 1. Effects of Diazepam (10 mg/70 kg, iv) and Scopolamine (0.5 mg/70 kg, iv) on Memory for Pictures*

	Number of Subjects	Per Cent of Pictures Forgotten Mean ± SE	Per Cent False Positive	Sedation Score (1 to 4) Mean
Test at 24 hours				
1. Ward control	31	5 ± 1.6	2	1.0
2. Operating room control	20	6 ± 1.9	0	1.0
3. Scopolamine	24	14 ± 3.2	1	—
4. Diazepam	26	41 ± 6.5	3	2.3
5. Diazepam + scopolamine	48	64 ± 5.3	2	2.3
Tests at 1 minute + 24 hours				
6. Ward control	21	4 ± 1.6	1	1.0
7. Diazepam	31	19 ± 3.4	2	1.9
8. Diazepam + scopolamine	44	33 ± 4.5	5	3.1
Tests at 1 minute + 24 hours, no amnesia at 1 minute				
6a. Ward control	20	4 ± 1.7	1	1.0
7a. Diazepam	20	15 ± 3.6	1	1.9
8a. Diazepam + scopolamine	20	28 ± 6.3	4	2.9

* The interval between drug administration and trial exposure to pictures was 15 minutes. The interval to the test exposure was 24 hours.

The present study attempts to show that diazepam and/or scopolamine could impair both registration and retention.

Methods

Patients between 15 and 60 years of age scheduled for elective surgical procedures served as subjects. Groups of 20 or more subjects were used for each drug relationship studied. The patients were studied in the holding area outside the operating room suite; none had received any drug that could affect the central nervous system, such as barbiturates, narcotics or anticholinergics, for at least 12 hours prior to study. Since the drugs that were administered are routinely used for premedication and have been approved by the Food and Drug Administration for this purpose and in this dosage range, consent was not requested. Because diazepam is available only as a microsuspension, not as a solu-

TABLE 2. Intergroup Comparisons of Amnesia for Pictures When Tested 24 Hours after Trial Exposure

		1	2	3	4	5	6	7	8	6a	7a	8a
1. Ward control	5%											
2. OR control	6%	N										
3. Scopolamine	14%	5	5									
4. Diazepam	41%	1	1	1								
5. Diazepam + scopolamine	64%	1	1	1	1							
6. Ward control (2)	4%											
7. Diazepam (2)	19%				1		1					
8. Diazepam + scopolamine (2)	33%					1	1	5				
6a. Ward control (20)	4%											
7a. Diazepam (20)	15%				1					1		
8a. Diazepam + scopolamine (20)	28%					1				1	1	

(2) = subjects exposed to two tests, *i.e.*, at 1 minute and 24 hours, in addition to the trial exposure.
(20) = subjects (2) above who had no amnesia during the test carried out 1 minute after the trial exposure.
1 = difference between the two groups significant ($P < 0.01$).
5 = difference between the two groups significant ($P < 0.05$).
N = difference between the two groups not significant ($P > 0.05$).

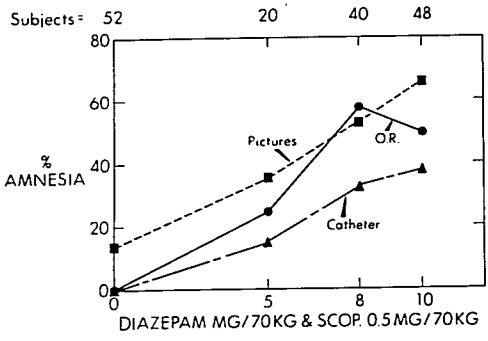

FIG. 1. Dose–response curves for three tests of memory. The doses of diazepam were 0, 5, 8, and 10 mg/70 kg. All subjects received in addition 0.5 mg/70 kg of scopolamine. The interval between intravenous injection of the drugs and the training display of pictures was 15 minutes. All subjects were tested again 24 hours later.

tion, all injections were made directly into a vein without any intervening plastic tubing. Diazepam, scopolamine, or both in rapid succession were administered intravenously within one minute. The dosages were adjusted according to weight and are expressed as mg/70 kg body weight unless otherwise designated. Diazepam was used in doses of 5–10 mg/70 kg and scopolamine in a dose of 0.5 mg/70 kg to a maximum of 0.5 mg. Fifteen minutes later, a training display was carried out: ten 8 × 10-inch pictures of emotionally "neutral" household items were displayed. The subjects identified them verbally. Subjects were not included if, because of drowsiness or any other reason, they could not identify all of the pictures at this time. Fifteen minutes after this display of pictures, a conventional large (16-gauge) plastic catheter used for intravenous therapy was inserted percutaneously in the forearm without local anesthesia. Rarely was the catheter insertion made as long as 30 minutes after picture display. The subject was then brought to the operating room and generally anesthesia was induced, usually with thiopental, although occasionally regional anesthesia was used, in which case the patient often remained awake. Twenty-four hours later, when the subjects had received no sedative or narcotic drug for at least six hours, they were tested by being shown 20 pictures, the original ten interspersed with ten similar pictures not shown previously. At the time of the test, the subjects were also asked whether they could remember the percutaneous insertion of the catheter and the operating room, *i.e.*, the gowned individuals, overhead lights, tables, etc. Administration of the drugs, presentation of the preanesthetic stimuli, and postanesthetic testing were almost invariably carried out by the same individual.

To evaluate impairment of registration and to establish a stringent criterion for intact registration at the time of trial, additional studies were performed in another group of subjects. The test displays were carried out twice, *i.e.*, initially one minute after the training display (16 minutes after drug injection) and then 24 hours later. If registration were entirely intact, then there should have been no memory loss in the first test carried out one minute after the training display.

Drowsiness or sedation at the time of trial was scored on a scale of 1 to 4: 1 represented subjects who were awake and alert; 2, awake, but drowsy; 3, drowsy but easily aroused; 4, drowsy and difficult to arouse. The ward controls were hospital patients who were not scheduled for operation, received no drug, and had a single trial of pictures and the percutaneous insertion of the catheter. The operating room controls were subjects who were operated on but who received no drug prior to induction of anesthesia.

For pictures, the summarizing constants for each group were N, the number of subjects, m, the mean, and SE, the standard error of the mean of the pictures forgotten. The extent of amnesia for pictures was assessed by first

scoring all failures of each individual to recognize, during a test display, any of the ten pictures shown during the original training display. The mean percentage of such failures together with the standard error of the mean was then computed for each group of subjects and constituted the amnesic score. The intergroup comparisons were made using the two-tailed z test for uncorrelated groups. The percentages of subjects who forgot the operating room and the catheter were calculated for each group. The intergroup comparisons for these two parameters were made using the X^2 statistics for the fourfold table and the Yates continuity correction. Differences between groups were considered significant at either the 1 per cent or the 5 per cent level ($P < 0.01$ or 0.05) or as not significant when P exceeded 5 per cent.

Results

Pictures—Tables 1 and 2, Figure 1

The summarizing constants (N, m and SE_m) for all groups of subjects are given in table 1, while the relevant intergroup comparisons are shown in table 2. The ward and operating room controls had negligible amnesia (5 and 6 per cent) and their difference was not significant. False-positive identification (*i.e.*, naming any of the ten "false" pictures shown only during the subsequent test displays) that might be considered "guessing" occurred only rarely, never exceeding 5 per cent. The amnesic scores were therefore not corrected for such false-positive identifications.

Scopolamine produced 14 per cent amnesia for the pictures which was different from amnesia in the two control groups (5 *vs.* 14 per cent; 6 *vs.* 14 per cent), as well as from the pooled controls. Diazepam produced marked, significant amnesia, 41 per cent. It also caused mild, variable sedation, but no undesirable side effect such as confusion or agitation. The combination of diazepam and scopolamine produced the highest amnesic score, 64 per cent, which was significantly greater than that for diazepam alone (64 *vs.* 41 per cent). Scopolamine, itself an amnesic agent, when in combination with diazepam adds significantly to the amnesic effect of diazepam alone.

The results for the subjects to whom two test displays were made are presented as two sets, *i.e.*, the group as a whole (6,7 and 8) and the subset consisting of subjects who showed no amnesia at the time of the one-minute test (6a, 7a and 8a). The latter subjects were those from the group as a whole who successfully identified one minute after the training display all of the pictures shown originally during the training display. As before, for the group as a whole, the amnesic effects of both diazepam and diazepam + scopolamine were significantly greater than their controls (4 *vs.* 19 per cent; 4 *vs.* 33 per cent). These differences are also significant when the subset is analyzed (4 *vs.* 15 per cent; 4 *vs.* 28 per cent). Likewise, scopolamine adds significantly to the amnesia caused by diazepam alone, both for the group as a whole (19 *vs.* 33 per cent) and for the subset (15 *vs.* 28 per cent).

The "learning" effect of introducing the one-minute test upon the picture scores of the 24-hour test is clearly shown. The amnesic scores for diazepam were significantly lower when there were two test displays compared with only one test display (19 *vs.* 41 per cent), and this effect was also observed in the subset with no amnesia at the one-minute test (15 *vs.* 41 per cent). Similar significant effects were seen when scopolamine was combined with diazepam (33 *vs.* 64 per cent; 28 *vs.* 64 per cent).

The combination of other doses of diazepam (5 and 8 mg/70 kg) and scopolamine (0.5 mg/70 kg) were used to establish a dose–response relationship between these combinations and amnesia. Figure 1 shows the virtually linear relationship between amnesic scores for pictures and increasing diazepam dosages.

Operating Room and Catheter (Table 3, Figure 1)

Scopolamine alone, surprisingly, did not produce any amnesia for these two stimuli, and thus resembled controls. Diazepam, however, produced 32 per cent amnesia for the operating room but only 9 per cent for the distinctly stronger stimulus, the percutaneous insertion of the catheter. The combination of diazepam and scopolamine was significantly more effective than diazepam alone, both for

TABLE 3. Effects of Diazepam (10 mg/70 kg, iv) and Scopolamine (0.5 mg/70 kg, iv) on Memory for the Operating Room and the Percutaneous Catheter

	Number of Subjects	Per Cent Forgotten		Sedation Score (1 to 4) Mean
		Operating Room	Catheter	
Ward control	52	—	0	1.0
Operating room control	23	0	0	1.0
Scopolamine	24	0	0	—
Diazepam				
1 test	26	38	12	2.3
2 test	30	29	7	1.9
Combined	56	34	9	2.1
Diazepam + scopolamine				
1 test	47	55	43	2.3
2 test	43	53	38	2.9
Combined	90	54	40	2.6

Diazepam *vs.* diazepam + scopolamine, combined groups: Operating room, 34 *vs.* 54 per cent; $P < 0.05$. Catheter, 9 *vs.* 40 per cent; $P < 0.01$.

the operating room (32 *vs.* 50 per cent) and for the catheter (9 *vs.* 38 per cent).

As with the pictures, combined intermediary doses of diazepam and scopolamine were used together with these data in constructing the dose–response curve describing these combinations and the amnesia for the operating room and the catheter (fig. 1). Although the relationship is clearly evident, the linearity is not as great as that seen with the picture scores.

Discussion

In evaluating this impairment of memory, it was important to differentiate between the effects of sedation, which could interfere with registration or short-term memory, and interruption of the consolidation process. Since all subjects were required to identify each picture at the training display, clearly some registration must have occurred at that time. This does not exclude the possibility that some impairment of registration also might have occurred. Therefore, a more stringent criterion of intact registration at the time of the trial exposure was then established by carrying out in an additional group of subjects a test for short-term memory one minute after the training display. As indicated previously, no memory loss would occur in the one-minute test if registration were intact at the time of the training display. The amnesic scores obtained in the one-minute tests were then used in conjunction with 24-hour scores of the same subjects. We considered that subjects who had 0 amnesic scores in the one-minute test had virtually intact registration, and consequently any amnesia they would display in the 24-hour test would have been the result of interfering processes occurring during retention and retrieval. Hence, our interpretation of these findings is that impairment of consolidation of the original short-term memory trace was responsible for the observed amnesia.

We considered that registration and immediate memory were virtually intact in subjects with 0 test scores at one minute for the reasons suggested above. However, it is possible that a more difficult test of memory would have avoided such a "ceiling" effect and might under those circumstances have shown some impairment of registration. Nevertheless, the criterion used here was clearly quite stringent. On the other hand, the increases in the amnesic scores, particularly for pictures, were accompanied by increases in mean sedation ratings. This finding supports the inference that some impairment of registration might likewise have taken place even though the performance by the other criterion (0 amnesia at one minute) indicated intact registration.

Clarke *et al.*,[18] using word retention, a vigilance test, and decision making, employed a post-trial intravenous dose of diazepam (15 mg/70 kg) to examine this point. They were

not able to demonstrate any retrograde amnesia in carefully performed and detailed studies designed to show such an effect if it had occurred. Grove-White and Kelman,[14] however, concluded that it is an impairment of consolidation that produces the amnesia under conditions in which only short-term memory is tested.

The test display at one minute in our two-test group must also be considered an additional learning experience, comparable to the initial trial display. Therefore, it was to be expected that the amnesic scores for diazepam and for diazepam + scopolamine were significantly lower than that obtained with only the single training display and the single test display at 24 hours.

Scopolamine increased slightly the amnesia for the pictures, but had no effect on memory for the operating room or the catheter. These results are somewhat surprising in view of the widely held position that scopolamine in therapeutically useful dosages is effective in producing amnesia,[19] but are consistent with the findings of Pandit and Dundee.[12] It is interesting that although scopolamine alone is rather weak as an amnesic agent, in combination with diazepam it apparently adds significantly to amnesic effect of diazepam.

It was important to determine at the outset whether the subsequent exposure to general anesthesia and operation might have an amnesic effect, albeit retrograde, was independent of the drugs we administered. However, the results with the ward and the operating room controls indicate that, under the conditions of this study, the events in the operating room apparently caused no retrograde amnesia.

The precise anatomic substrate, if any, for a memory trace has not yet been demonstrated unequivocally, nor has this study defined the mechanism or site of action of diazepam—alone or in combination with scopolamine—in producing amnesia. However, considerable evidence from both ablation and stimulation studies implicates the limbic system in the consolidation of the short-term memory trace.[20] Furthermore, diazepam has been shown to depress the limbic system.[21] Therefore, it may be that limbic system depression accounts for the amnesia seen in this study. Alternatively, it is now well established that the brain-stem reticular activating system is intimately concerned with the maintenance of the waking state,[22] and thus may also be implicated in memory. Przybyla and Wang[23] have shown that diazepam alters spinal reflexes by depressing this reticular activating system. It is therefore also conceivable that reticular depression by diazepam plays a role in producing amnesia. Adam[24] has shown that inhalation of low concentrations of anesthetic drugs—often assumed to depress the reticular formation—also produces amnesia.

Pandit, Dundee and Keilty[25] studied the amnesic effects of a large number of drugs alone and in various combinations, including scopolamine, diazepam and their combinations, but with higher dosages and under somewhat different test conditions. In general, our results are similar to their findings for postoperative memory of the catheter. However, they used grosser criteria and did not use multiple stimuli, as we did when we employed pictures, a technique that allows a finer analysis of this graded response. Dundee and Pandit[26] tested scopolamine and diazepam, but used a considerably different experimental design and had various other objectives. Their general conclusion was that these two drugs, studied independently, do produce amnesia. However, they did not study, as we did, the effects of combined administration. The present study indicates that diazepam and scopolamine can each produce not only impairment of learning, but accelerated forgetting. The combination of the two drugs produces results greater than those found with each drug alone. It would be worthwhile to examine combinations of this type, and perhaps even three-drug combinations, in order to eliminate some of the side effects unrelated to memory loss, such as delirium, and peripheral anticholinergic effects. Accelerated forgetting can be interpreted as amnesia or impairment of consolidation if one assumes that such forgetting is due to the inability of memory to transfer from short-term to long-term storage.

References

1. Gauss CJ: Geburten in kunstlichem Dämmerschlaff. Arch Gynaekol 78:579–631, 1906
2. Bohdanecky Z, Jarvik ME: Impairment of one trial learning in mice by scopolamine, scopolamine methylbromide and physostig-

mine. Int J Neuropharmacol 6:217–222, 1967
3. Carlton P: Cholinergic mechanisms in the control of behavior. Psychopharmacology. Edited by Efron DH. USPHS Publ 1836, 1968, pp 125–138
4. Deutsch JA: The physiological basis of memory. Annu Rev Psychol 20:85–104, 1969
5. Orkin LR, Bergman PS, Nathanson M: Effects of atropine, scopolamine and meperidine on man. ANESTHESIOLOGY 17:30–37, 1956
6. Longo VG: Behavioral and electroencephalographic effect of atropine and related compounds. Pharmacol Rev 18:965–996, 1966
7. Hardy TK, Wakely D: The amnesic properties of hyoscine and atropine in pre-anaesthetic medication. Anaesthesia 17:331–336, 1962
8. Haslett WHK, Dundee JW: Studies of drugs given before anaesthesia. XIV: Two benzodiazepine derivatives—chlordiazepoxide and diazepam. Br J Anaesth 40:250–257, 1968
9. Brandt AL, Oakes FD: Preanesthesia medication: Double blind study of new drug, diazepam. Anesth Analg (Cleve) 44:125–129, 1965
10. Nutter DO, Massumi RA: Diazepam in cardioversion. N Engl J Med 273:650–651, 1965
11. Dundee JW, Haslett WHK, Keilty SR, et al: Studies of drugs given before anaesthesia. XX: Diazepam-containing mixtures. Br J Anaesth 42:143–150, 1970
12. Pandit SK, Dundee JW: Preoperative amnesia. Its incidence following the intramuscular injection of commonly used premedicants. Anaesthesia 25:493–499, 1970
13. Brown SS, Dundee JW: Clinical studies of induction agents. XX: Diazepam. Br J Anaesth 40:108–112, 1968
14. Grove-White IG, Kelman GR: Effect of methohexitone, diazepam and 4 hydroxybutyrate on short-term memory. Br J Anaesth 43: 113–116, 1971
15. Greenblatt DJ, Shader RI: Benzodiazepines in Clinical Practice. New York, Raven Press, 1974
16. Denisenko PP: Effects of central cholinolytics on the higher nervous activity of healthy people. Trans Inst Exp Med (Acad Med Sci) USSR 5:82–86, 1960
17. Barondes SH: Effects of inhibitors of central protein synthesis on long-term memory in mice. Psychopharmacology. Edited by Efron DH. USPHS Publ 1836, 1968, pp 905–908
18. Clarke PRF, Eccersley PS, Frisby JP, et al: The amnesic effect of diazepam (Valium). Br J Anaesth 42:690–697, 1970
19. Innes IR, Nickerson M: Drugs inhibiting the action of acetylcholine on structures innervated by postganglionic parasympathetic nerves (antimuscarinic or atropinic drugs). The Pharmacological Basis of Therapeutics. Fifth edition. Edited by Goodman LS, Gilman A. New York, Macmillan, 1975, p 517
20. Whitty EWM, Zangwill OL: Amnesia. London, Butterworths, 1966
21. Morillo A: Effects of benzodiazepines upon amygdala and hippocampus of cat. Int J Neuropharmacol 1:353–359, 1962
22. Moruzzi G, Mogoun HW: Brain stem reticular formation and activation of the EEG. Electroenceph Clin Neurophysiol 1: 455–473, 1949
23. Przybyla AC, Wang SC: Locus of central depressant action of diazepam. J Pharmacol Exp Ther 163:439–447, 1968
24. Adam N: Effects of general anesthetics on memory functions in man. J Comp Physiol Psychol 83:294–305, 1973
25. Pandit SK, Dundee JW, Keilty SR: Amnesia studies with intravenous premedication. Anaesthesia 26:421–428, 1971
26. Dundee JW, Pandit SK: Anterograde amnesic effects of pethidine, hyosine and diazepam in adults. Br J Pharmacol 44:140–144, 1972