Pharmacology Biochemistry and Behavior, Vol. 41, pp. 573–579, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Effect of a New Anxiolytic, DN-2327, on Learning and Memory in Rats

TAKEO WADA[1] AND NAOHISA FUKUDA

Biology Research Laboratories, Research and Development Division, Takeda Chemical Industries Ltd. Yodogawa-ku, Osaka 532, Japan

Received 16 September 1991

WADA, T. AND N. FUKUDA. *Effect of a new anxiolytic, DN-2327, on learning and memory in rats.* PHARMACOL BIOCHEM BEHAV **41**(3) 573–579, 1992.—The effects of a new anxiolytic, (2-(7-chloro-1,8-naphthyridin-2-yl)-3-[(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-carbonylmethyl] isoindolin-1-one (DN-2327), on the execution of step-through passive avoidance and delayed spontaneous alternation tasks were assessed and compared with those of diazepam (DZP) and buspirone. DN-2327 and buspirone (both 10 and 20 mg/kg, PO) impaired performance in the 48-h passive avoidance recall test when given prior to the test session, but not when given before the training trial. DZP impaired the performance at doses of more than 5 and more than 10 mg/kg PO when given prior to the test session and when given before the training trial, respectively. The action of DZP (10 mg/kg, PO) when given before the training trial was antagonized by flumazenil (20 mg/kg, IP) and tended to be antagonized by DN-2327 (10 and 30 mg/kg, PO), but was not affected by buspirone. No evidence for possible amnesic effects of DN-2327 or buspirone on working memory was found in the delayed spontaneous alternation task, but DZP (3 and 10 mg/kg, PO) caused significant impairment of working memory. Electroshock sensitivities detected by flinch, jump, and vocalization thresholds were not influenced significantly by DN-2327 (30 and 100 mg/kg, PO), DZP (10 and 30 mg/kg, PO) or buspirone (30 and 100 mg/kg, PO). These results suggest that although DN-2327 and buspirone have amnesic effects in tasks that involve anxiety they do not impair acquisition or working memory, both of which are impaired by DZP, and that DN-2327 tends to act as an antagonist on the acquisition impairment caused by DZP in the passive avoidance task.

Learning and memory Anxiolytic DN-2327 Diazepam Buspirone Passive avoidance
Delayed spontaneous alternation response Rat

THE benzodiazepines (BZDs), represented by diazepam, are the class of drugs most frequently as pharmacotherapeutics for anxiety-related disorders. Certain behavioral effects of these medications, however, may cause problems in some patients; high doses of BZDs generally cause drowsiness and impair psychomotor performance tasks when initially administered and potentiate the behavior-impairing effects of other CNS depressants such as alcohol (22,27). Moreover, BZD medications may impair memory function by causing short-term anterograde amnesia (6,12,16,18,31). Such an effect may be considered desirable when these drugs are used in anesthesia, and probably does not represent an important side effect for their use as sleep inducers, unless there are residual effects following a night of drug-induced sleep. However, this effect of BZDs might be considered undesirable if they are prescribed for alleviating daytime anxiety.

The memory impairment caused by BZDs is potentially troublesome for two reasons: first, patients are pharmacologically more sensitive to this effect than other behavioral side effects (5); second, complete tolerance to the memory-impairing effect of BZDs does not develop with repeated administration (19,21).

Animal experiments have also shown that BZDs can interfere with learning and memory in passive avoidance tasks (3,34), radial maze performance (14), and the Morris water task (24). Since memory impairment is caused at almost the same dose as the anxiolytic effects of BZDs (5), it is also thought that these amnesic and anxiolytic effects may be non-duplicate effects in the same dimension.

On the other hand, it has been suggested that the 5-hydroxytryptamine (5-HT) receptor 5-HT_{1A} partial agonist, buspirone, may cause less impairment of behavioral performance than equivalent doses of diazepam but with the same anxiolytic action (2,17). In addition, performance impairment caused by alcohol is potentiated by diazepam but not by buspirone (22,27), and buspirone has been reported not to impair memory function in subjects with generalized anxiety disorder (20). It has therefore been suggested that the anxiolytic effect may be separated from the memory-impairing effect.

[1] Requests for reprints should be addressed to T. Wada, Biology Research Laboratories, Research and Development Division, Takeda Chemical Industries Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.

(2-(7-chloro-1,8-naphthyridin-2-yl)-3-[(1,4-dioxa-8-aza-spiro [4.5]dec-8-yl)-carbonylmethyl] isoindolin-1-one (DN-2327), a novel isoindoline derivative, produces anticonflict, taming, and anticonvulsive effects when administered orally to several species of animals (39), but produces few of the sedative-hypnotic and muscle-relaxant effects observed with diazepam. DN-2327 shows potent displacement activity against [^{3}H]diazepam binding. The binding affinity of DN-2327 for BZD receptors is not enhanced in the presence of GABA. Furthermore, behavioral assessment has shown that DN-2327 differentially influences BZD receptors: It acts as an agonist on the anticonflict and anticonvulsive effects but as an antagonist on the muscle-relaxant and sedative effects of diazepam, that is, DN-2327 possesses mixed agonist/antagonist properties (38). For compounds to be used to alleviate daytime anxiety, it is important to determine whether or not they cause any memory impairments. The present experiment focused on the effect of DN-2327 on learning and memory in comparison with diazepam and buspirone.

Up to now, most previous studies concerning the effects of anxiolytics on learning and memory in animals have employed tests related to not only learning and memory but also anxiety. For example, Rowan et al. (33) recently reported that buspirone induced an impairment of spatial learning in the Morris water maze using rats. However, as there is a possibility that buspirone could have reduced the aversion to the stimulus, soaking in the water, it seems difficult to definitely conclude that buspirone caused learning impairment.

In the present study, we subjected rats to passive avoidance and delayed spontaneous alternation tasks to assess the effects of anxiolytics on learning and memory. Although an electrical aversive stimulus was used for the passive avoidance task, the effects of anxiolytics on the acquisition process were examined separately from the effects on the recall process, and considering that these anxiolytics may generally reduce sensitivity to aversive stimuli the test trial was done 48 h after the acquisition trial, rather than 24 h after, to allow clearance of the drugs. The effects on working memory were examined using a delayed spontaneous alternation task, without any aversive stimuli, with a food reward.

METHOD

Passive Avoidance Learning

Animals. Male rats (Jcl: Wistar, 210–261 g, 8 wk old, n = 12–18 in each group) were used in all experiments. They were housed individually from the acquisition trial to the recall test (2 days) and allowed free access to food and water. They were kept in an air-conditioned room with controlled temperature (23–25°C), humidity (50–60%), and lighting (lights on from 0730–1930).

Apparatus. Rats were trained in a conventional step-through-type passive avoidance training box divided into two compartments (an illuminated side measuring 12 × 12 × 28 cm and a dark side measuring 12 × 31 × 28 cm) by a sliding door with an opening (12 × 9 cm) 3 cm above the floor. The safe compartment had a flat floor and was illuminated by a 15-W fluorescent lamp located 1 cm outside a transparent wall. The dark compartment, painted black, had a grid floor made of 2-mm wide stainless steel rods, spaced 1.1 cm apart, used to give a foot-shock that was delivered by means of a scrambled DC constant-current shock generator (SEIKO DENKI, ES2010). The experimental room was kept as dark as possible, and masking white noise was generated throughout the experiment.

Procedure

Effects on the acquisition process. The experimental procedure used was similar to that described by Yamazaki et al. (40). Rats were trained in a one-trial passive avoidance task. In the habituation trial, 5 s after the animal had been placed in the safe compartment the door was opened and the time (latency) elapsed before the animal stepped through the door into the dark compartment was measured. When all four paws were on the floor in the dark compartment, the door was closed; rats were removed from the dark compartment 5 s later and then drugs were administered orally. An acquisition trial was carried out 30–60 min later in the same way except that an inescapable foot-shock (2 mA, 3 s) was delivered when the rat entered the dark compartment.

In the test trial 48 h after the acquisition trial when presumably no drug remains in the body, the rat was again placed in the safe compartment and the latency for the animal to enter the dark compartment was measured. Animals that did not enter the dark compartment within 300 s were assigned a ceiling score of 300 s.

Effects on the recall process. Drugs were not administered before the acquisition trial but 1 h before the recall test. The other procedures were the same as above.

Determination of Nociceptive Threshold

Animals. Male rats (Jcl: Wistar, 241–267 g and 8 wk old, n = 8–9 in each group) were used in all experiments. They were housed in groups of five and allowed free access to food and water. The environmental conditions and light/dark cycle were the same as mentioned above.

Apparatus. The test chamber (30 × 30 × 30 cm) was made of vinyl chloride and equipped with a grid floor of stainless steel that was connected to a scrambled electric shock generator (Grason-Stadler, GSC-700) modified to supply an output current below 0.1 mA. Note that this electric shock generator is different from that used in passive avoidance task.

Procedure. Each rat was placed in the test chamber and given a 1-min period for habituation before application of a shock. A series of inescapable shocks was then delivered to the grid floor. Each series consisted of 11 stimulations at the following shock intensities (mA): 0.1, 0.13, 0.16, 0.2, 0.25, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0. Shocks were presented in ascending order for 1 s at 10-s intervals. A "flinch" was defined as a form of startle behavior in which the animal's paws did not leave the floor and a "jump" as removal of three or more paws from the grid floor. The shock intensity at which rats exhibited each response was measured and considered the threshold.

Delayed Spontaneous Alternation Task

Animals. Male rats (Jcl: Wistar, 8 wk old, n = 30) were trained and finally 18 rats, which showed more than an 80% correct response in three consecutive sessions, were used for the test. They were housed individually and partially deprived of food so as to maintain their body weight between 260 and 310 g. Water was constantly available in the home cage. The environmental conditions and light/dark cycle were the same as mentioned above.

Apparatus. The apparatus was a gray T-maze made of vinyl chloride with 12-cm high and 12-cm wide alleys with a clear acrylic resin top. The starting box, the runway, and each arm of the maze were 12, 44, and 50 cm long, respectively.

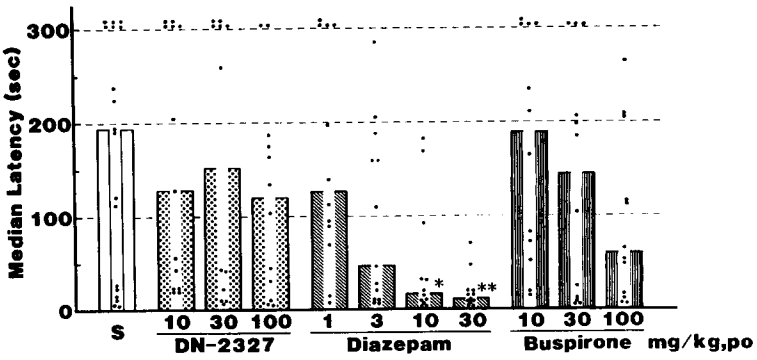

FIG. 1. Effects of DN-2327, DZP, and buspirone on 48-h recall of a step-through passive avoidance task when administered orally to rats (n = 12–18) 60 min prior to the training (acquisition) trial. Values are expressed as median step-through latency (s). *$p < 0.05$, **$p < 0.01$, vs. saline (S) control (Mann–Whitney U-test).

The floor of the maze was a grid of 2-mm stainless steel rods spaced 1 cm apart. A food cup, 5 mm deep and 1 cm in diameter, was placed 3 cm above the floor in the middle at the end of each arm. A gray guillotine door was mounted at the entrance of the runway. White noise was generated throughout the experiment.

Procedure. After the rats had been deprived of food and familiarized well with food pellets (45 mg each) in the home cage for 1 week, they were acclimatized to the T-maze apparatus with the cups filled with food pellets for 5 min. Over the next 5 days, each animal received 11 training trials per day. In each trial, the rat was placed in the starting box and then allowed to run to one of the food-filled cups after the guillotine door had opened. In the first trial, both cups were filled with food pellets. However, from the second to the eleventh trial the cup that was not selected just before the trial was alternately filled with a food pellet. The intertrial interval was made as short as possible. A correct response was defined as selection of the food-filled cup from the second to the eleventh trial. This training finally enabled rats to arrive at a food cup within 3–5 s. Finally, 18 rats, which showed more than an 80% correct responses in three consecutive sessions, were used for the following tests. First, the effects of the intertrial intervals were examined at intervals of 15, 30, 60, and 120 s, and successively the effects of the anxiolytics were examined at a 30-s intertrial interval twice a week (Tuesday and Friday). One day before the test (Monday and Thursday), rats were trained to respond correctly for five consecutive trials without any intervals.

The 18 rats were divided into 9 groups and each rat was treated with 9 drug conditions at 3- or 4-day intervals to counterbalance the effect of the order of administration.

Drugs. The following drugs were used: DN-2327 (Takeda) (39), diazepam (Cercine, Takeda), buspirone HCl (Buspar, Bristol Myers), and flumazenil (Hoffmann-La Roche). DN-2327, diazepam, buspirone HCl, and flumazenil were suspended in 5% gum arabic saline. DN-2327, diazepam, and buspirone were administered orally 60 min before the test and flumazenil was administered intraperitoneally 30 min before the test. Drugs and saline were administered in a volume of 2 ml/kg body weight.

Statistical analysis. In the passive avoidance test, the two-tailed Mann–Whitney U-test was used to analyze the median latency, and in the other experiments, analysis of variance (ANOVA) and a two-tailed paired t-test were used.

RESULTS

Passive Avoidance Learning

Effects on the acquisition process. When drugs were administered only before the acquisition trial in the passive avoidance task, neither DN-2327 nor buspirone (10, 30, or 100 mg/kg, PO) affected the latency 48 h after drug administration. On the other hand, the latency was shortened dose dependently by DZP at doses of 1, 3, 10, and 30 mg/kg PO and it was significantly different from that in the saline control at 10 and 30 mg/kg PO DZP [$F(12,18) = 48.0$, $p < 0.05$, and $F(12,18) = 35.0$, $p < 0.01$, respectively] (Fig. 1).

The latency for the rat to enter the dark room in the acquisition trial was significantly lengthened by 100 mg/kg PO buspirone, $F(12,18) = 11.5$, $p < 0.01$. This dose of buspirone seemed to be an overdose as it induced diarrhea in 7 of 12 rats.

Effects of flumazenil, DN-2327, and buspirone on diazepam-induced acquisition impairment. The BZD receptor an-

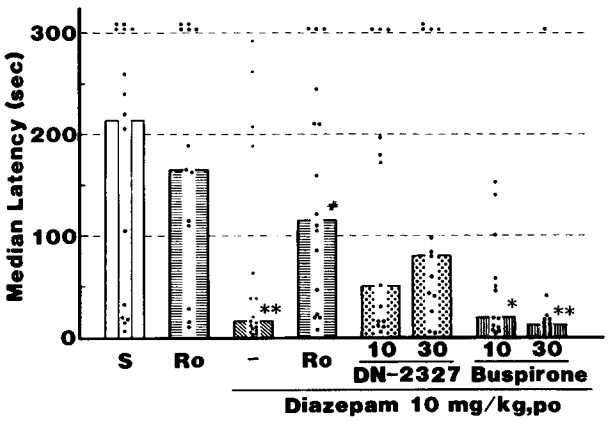

FIG. 2. Effects of flumazenil, DN-2327, and buspirone on the action of diazepam administered orally 60 min prior to the training trial in the passive avoidance task (n = 13–17). DN-2327 and buspirone were administered orally 60 min prior to and flumazenil (Ro) was administered intraperitoneally 30 min prior to the training trial. Values are expressed as median step-through latency (s). *,#$p < 0.05$, **$p < 0.01$, vs. saline- and diazepam-treated groups, respectively (Mann–Whitney U-test).

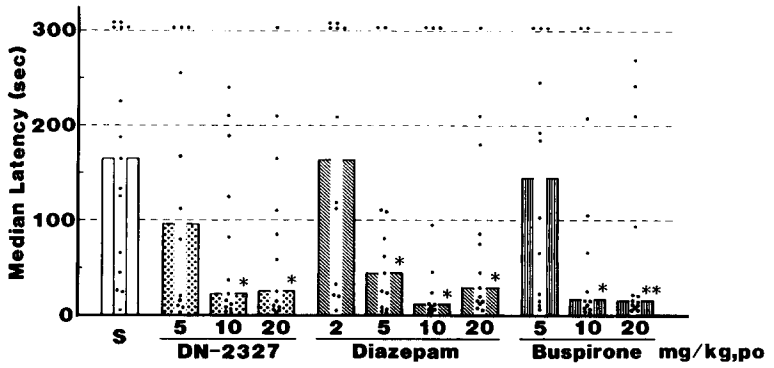

FIG. 3. Effects of DN-2337, DZP, and buspirone on 48-h recall of a step-through passive avoidance task when administered orally to rats (n = 12-15) 60 min prior to the test trial. Values are expressed as median step-through latency (in seconds). *$p < 0.05$, **$p < 0.01$, vs. saline (S) control (Mann-Whitney U-test).

tagonist, flumazenil, at 20 mg/kg IP significantly lengthened the test latency, which was shortened by 10 mg/kg PO DZP given before the acquisition trial $F(16,17) = 67.0$, $p < 0.05$, that is, flumazenil attenuated the DZP-induced impairment of acquisition learning. DN-2327 at doses of 10 and 30 mg/kg PO given concomitantly with 10 mg/kg PO DZP, tended to antagonize dose dependently the DZP-induced acquisition impairment [$F(13,17) = 65.0$, $0.05 < p < 0.1$ at 30 mg/kg PO]. However, buspirone at the same doses as DN-2327 did not show such an effect (Fig. 2).

Effects on the recall process. When drugs were administered only before the recall trial 48 h after acquisition learning of the passive avoidance task, the latency was shortened dose dependently by DN-2327 and buspirone (5, 10, and 20 mg/kg, PO), and it was significantly different from that of the saline control with either drug at doses of 10 and 20 mg/kg PO [$F(13,15) = 45.0$, $p < 0.05$, and $F(13,15) = 50.5$, $p < 0.05$, respectively at 10 mg/kg, PO] (Fig. 3).

Effects on the nociceptive threshold. As shown in Table 1, the threshold for shock intensities required to induce flinch, jump, or vocalization behavior was not affected by any of the doses of DN-2327 or diazepam used in the present experiments.

The sensitivity was decreased slightly by 100 mg/kg PO buspirone, although statistical analysis (ANOVA) indicated no significant differences.

TABLE 1

EFFECTS OF DN-2327, DZP, AND BUSPIRONE ON SHOCK INTENSITY THRESHOLDS (mA) IN RATS

Drug	Dose (mg/kg, PO)	Flinch	Jump	Vocalization
Saline	–	0.20 ± 0.02	0.49 ± 0.04	0.50 ± 0.08
DN-2327	30	0.21 ± 0.01	0.49 ± 0.03	0.55 ± 0.08
	100	0.20 ± 0.01	0.47 ± 0.02	0.54 ± 0.04
Diazepam	10	0.21 ± 0.01	0.53 ± 0.03	0.52 ± 0.05
	30	0.22 ± 0.01	0.51 ± 0.03	0.54 ± 0.04
Buspirone	30	0.21 ± 0.01	0.48 ± 0.03	0.45 ± 0.03
	100	0.23 ± 0.01	0.56 ± 0.03	0.57 ± 0.04

Sensitivity to shock was determined 1 h after drug administration. Values are expressed as means ± SEM (mA) for eight or nine rats.

Delayed Spontaneous Alternation Task

The effects of delayed time (0, 15, 30, 60, and 120 s) on correct responses were assessed (Fig. 4). The percentage of correct responses was time dependently reduced and asymptotically approached the chance level (50%), $F(4,68) = 14.273$, $p < 0.01$. The percentage of correct responses at a delayed time of more than 30 s was significantly different from that of nondelayed responses [$t(1,7) = 4.415$, $p < 0.01$, paired t-test].

The effects of drugs on working memory were examined using a 30-s delay time (Fig. 5). The percentage of correct responses was not influenced significantly by DN-2327 or buspirone (10 or 30 mg/kg, PO). However, DZP dose dependently reduced the percentage of correct responses (1, 3, and 10 mg/kg, PO) and the change was found to be significant at more than 3 mg/kg PO, $F(8,136) = 3.359$, $p < 0.01$.

DISCUSSION

In this experiment, the effects of new anxiolytic, DN-2327, on the execution of step-through passive avoidance and delayed spontaneous alternation tasks were assessed and compared with those of DZP, reported to impair memory function by causing short-term anterograde amnesia during therapeutic treatment, and buspirone, which produces no such effect.

Considering that these anxiolytics may generally reduce sensitivity to aversive stimuli, their effects on acquisition and recall processes were examined separately in the passive avoidance test. The test trial was done 48 h after the acquisition trial, rather than 24 h after, to allow clearance of the drugs (data not shown).

DN-2327 and buspirone (both 10 and 20 mg/kg, PO) impaired performance in the 48-h passive avoidance recall test when given prior to the test session, but not when given before the training trial. DZP impaired the performance at doses of more than 5 and more than 10 mg/kg PO when given prior to the test session and when given before the training trial, respectively. The action of DZP (10 mg/kg, PO) when given before the training trial was antagonized by flumazenil (20 mg/kg, IP). This suggested that the acquisition impairment caused by DZP occurred via BZD receptors. Moreover, it tended to be antagonized dose dependently by DN-2327 (10 and 30 mg/kg, PO) but was not affected by buspirone. We reported previously that DN-2327 possesses mixed agonist/antagonist properties (38). The present results suggest that

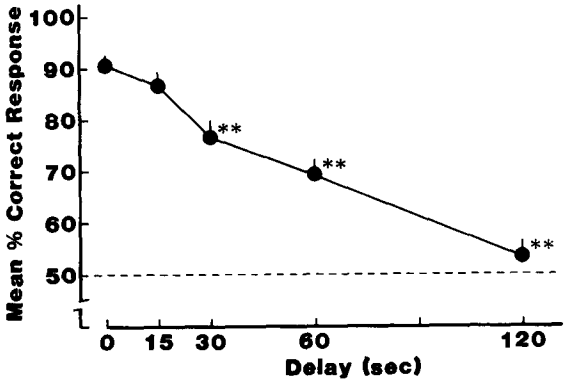

FIG. 4. Effect of the intertrial interval on the spontaneous alteration task using a T-maze and rats. Rats (n = 18) were tested under 5 delay conditions for 5 days (11 trials per day). Each point in the figure represents the mean percentage of correct responses for 180 observations. **$p < 0.01$ vs. no delayed control (ANOVA and paired t-test).

DN-2327 may act as an antagonist on the impairment of acquisition learning caused by DZP. The failure of buspirone to act as an antagonist may be due to differences between its mechanism of action and that of DZP.

Traber et al. (36) and Rowan et al. (33) suggested that anxiolytics may reduce fear or sensitivity to aversive stimuli in the drugged state because of their intrinsic anticonflict activity. It has also been reported from human studies that BZDs unequivocally impair acquisition or encoding of new information in episodic memory while having no disruptive effect upon either retention or recall of previously stored information (6,7). Moreover, it has been found that BZDs not only lack a retrograde amnesic effect but in many cases actually improve the retention of material learned before treatment (4,13,32). This phenomenon, known as retrograde facilitation of memory, is not thought to be the result of improved consolidation of the memory trace but rather a consequence of reduced learning of information presented after the drug takes effect (13,29). These findings suggest that recall impairment in a drugged state during the passive avoidance task may not reflect anterograde amnesia, which is a problem in human studies, but may be attributed to the anticonflict activity of the anxiolytics.

A relatively large component of the classical inhibitory effect of BZDs on the performance of passive avoidance tasks is known to be due to a state-dependent phenomenon, which means that memory acquired in a drugged state can be recalled in the same drugged state but not in an undrugged state [(1,30), but see (34)]. There is a possibility that state-dependent learning played a major role in producing DZP-induced deficits in performance of the passive avoidance learning task. Although the effect of DZP given before both acquisition and recall testing was preliminarily examined, state-dependent learning of DZP could not be detected in this task. DZP given before the recall testing may have induced anticonflict activity rather than state-dependent learning of DZP. However, it appears at least that DN-2327 and buspirone do not induce state-dependent learning or acquisition impairment.

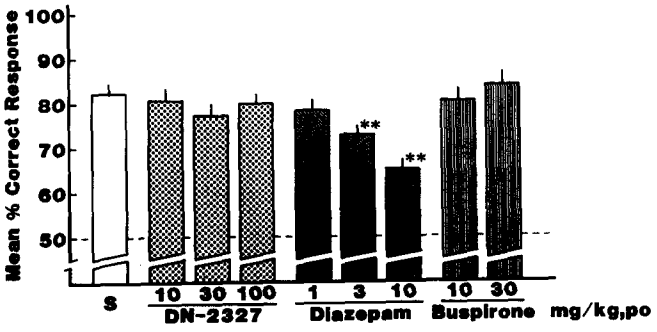

FIG. 5. Effect of DN-2327, DZP, and buspirone on the 30-s delayed spontaneous alternation task using rats (n = 18). Drugs were given orally 60 min prior to the first trial. **$p < 0.01$ vs. saline (S) control (ANOVA and paired t-test).

The latency observed at the acquisition trial may involve the effects of drugs on motivation or locomotor activity because foot-shock has not yet been delivered at that time. In the present study, the highest dose (100 mg/kg, PO) of DN-2327 or 30 mg/kg PO DZP did not affect the latency at the acquisition trial, whereas buspirone at 100 mg/kg PO prolonged it. However, as the drugs should have been excreted 48 h after administration, their effects on motivation or locomotor activity need not be considered when looking at the results of latency at the recall trial. Moreover, the electroshock sensitivity detected by flinch, jump, and vocalization thresholds was not influenced significantly by DN-2327 (30 and 100 mg/kg, PO), DZP (10 and 30 mg/kg, PO), or buspirone (30 and 100 mg/kg, PO). Consequently, the impairment of acquisition learning in this passive avoidance task may not be involved in electroshock sensitivity. These results are in agreement with a report that BZD anxiolytics impair acquisition learning at doses that do not affect movement or electroshock sensitivity in passive avoidance learning (30).

In the delayed spontaneous alternation task, which does not involve any aversive stimuli, the memory impairment induced by anxiolytics can be examined separately from anxiolytic effects. The percentage of correct responses was reduced according to the delay time (0, 15, 30, 60, and 120 s) and asymptotically approached the chance level, implying that the retention of working memory becomes more and more difficult with increased time delay. The percentage of correct responses at a delay time of more than 30 s was significantly different from that with no delay time. No evidence was found for a possible amnesic effect of DN-2327 or buspirone on working memory in the 30-s-delayed spontaneous alternation task, but DZP (3 and 10 mg/kg, PO) significantly impaired working memory.

Although it is well known that scopolamine impairs working memory, BZDs do not induce the same type of memory impairment as scopolamine. Scopolamine and DZP induce retention and acquisition impairment, respectively, as scopolamine does not cause memory impairment under nondelayed conditions but does do so under delayed conditions, whereas DZP has this effect under both conditions (26,37). Moreover, it has even been suggested that BZDs induce general disruption of information processing rather than specific impairment of working memory (14,35). In this experiment, although the effects of anxiolytics under nondelayed conditions were not examined it is thought that DN-2327 or buspirone does not bring about the same acquisition impairment as that caused by DZP or the retention impairment similar to that caused by scopolamine because neither DN-2327 nor buspirone induced any memory impairment under delayed conditions.

These results suggest that although DZP, DN-2327, and buspirone exerted amnesia-like effects in some of the tasks that involved anxiety neither DN-2327 nor buspirone impaired acquisition or working memory, both of which were impaired by DZP, and that DN-2327 tended to act as an antagonist of the acquisition impairment caused by DZP in the passive avoidance task.

Hashimoto et al. (11) compared the behavioral and EEG changes induced by DN-2327 with those induced by DZP and buspirone in cats. The frontal and parietal cortical EEG was altered by DN-2327, DZP, and buspirone. The power of low-frequency bands was decreased and that of high-frequency bands was increased, as seen with DZP. However, the power of the theta band of the hippocampal EEG during wakefulness or the peak frequency of its power density spectrum was not changed by DN-2327, although both were decreased by DZP. Buspirone, like DZP, decreased the power of the theta band of the hippocampal EEG, but did not change the peak frequency (11). It has also been reported that hippocampal theta waves were desynchronized by DZP but not by buspirone in the EEG of rabbits (15). It is well known that the hippocampus is related to learning and memory (9,24,28) and that its theta waves on EEG may reflect wakefulness, attention, or learning (8,10,23). It is of considerable interest that memory impairment was induced by DZP, which desynchronizes the hippocampal theta waves, but not by DN-2327 or buspirone, which have no such effect, even though different kinds of animals were used. This hypothesis is consistent with findings that not only BZDs but also anticholinergic drugs interfere with the production of hippocampal rhythmic slow activity (25). It has also been reported that memory impairment induced by chlordiazepoxide and anticholinergic drugs resembles that induced by septal and hippocampal lesions (9,35). These and the present investigations suggest that the memory impairment, and not the anxiolytic effects, produced by anxiolytics may depend on hippocampal activity. Further investigations will be necessary to confirm this hypothesis.

ACKNOWLEDGEMENTS

The excellent technical assistance of F. Wada, K. Ikeda, and H. Nishikawa is gratefully acknowledged.

REFERENCES

1. Berger, S. D.; Stein, L. Asymmetrical dissociation of learning between scopolamine and Wg4036, a new benzodiazepine tranquilizer. Psychopharmacologia 14:351–358; 1969.
2. Bond, A.; Lader, M.; Shrotriya, R. Comparative effects of a repeated dose regime of diazepam and buspirone on subjective ratings, psychological tests and the EEG. Eur. J. Clin. Pharmacol. 24:463–467; 1983.
3. Cole, S. O. Effects of benzodiazepines on acquisition and performance: A critical assessment. Neurosci. Biobehav. Rev. 10:265–272; 1986.
4. Dorow, R.; Berenberg, D.; Duka, T.; Sauerbrey, N. Amnestic effects of lormetazepam and their reversal by the benzodiazepine antagonist Ro15-1788. Psychopharmacology (Berl.) 93:507–514; 1987.
5. Ghoneim, M. M.; Hinrichs, J. V.; Mewaldt, S. P. Dose-response analysis of the behavioral effects of diazepam: I. Learning and memory. Psychopharmacology (Berl.) 82:291–295; 1984.
6. Ghoneim, M. M.; Mewaldt, S. P. Effects of diazepam and scopolamine on storage, retrieval and organizational processes in memory. Psychopharmacologia 44:257–262; 1975.
7. Ghoneim, M. M.; Mewaldt, S. P. Benzodiazepines and human memory: A review. Anesthesiology 72:926–938; 1990.
8. Gray, J. A.; Ball, G. G. Frequency-specific relation between hippocampal theta rhythm, behavior and amobarbital action. Science 168:1246–1248; 1970.
9. Gray, J. A.; McNaughton, N. Comparison between the behavioural effects of septal and hippocampal lesion: A review. Neurosci. Biobehav. Rev. 7:119–188; 1983.
10. Green, J. D.; Arduini, A. A. Hippocampal electrical activity in arousal. J. Neurophysiol. 17:533–557; 1954.
11. Hashimoto, T.; Wada, T.; Fukuda, N. Behavioral and EEG effects of novel anxioselective anxiolytic, DN-2327, in cats, 17th CINP Congress Meet. Abstr. 1:179p; 1990.
12. Heisterkamp, D. V.; Cohen, P. J. The effects of intravenous premedication with lorazepam (Ativan), pentobarbitone or diazepam on recall. Br. J. Anesth. 47:79–81; 1975.
13. Hinrichs, J. V.; Ghoneim, M. M.; Mewaldt, S. P. Diazepam and memory: Retrograde facilitation produced by interference reduction. Psychopharmacology (Berl.) 84:158–162; 1984.
14. Hodges, H.; Green, S. Effects of chlordiazepoxide on cued radial maze performance in rats. Psychopharmacology (Berl.) 88:460–466; 1986.
15. Kawasaki, H.; Nakamura, S.; Takasaki, K. Electroencephalographic study with buspirone, a novel nonbenzodiazepine anxiolytic, and its major metabolite, 1-(2-pyrimidinyl)piperazine (1-PP), in rabbits. Folia Pharmacol. Japonica 95:91–104; 1990 (in Japanese).
16. Kothary, S. P.; Brown, A. C. D.; Pandit, U. A.; Samara, S. K.; Pandit, S. K. Time course of antirecall effect of diazepam and lorazepam following oral administration. Anesthesiology 55:641–644; 1981.
17. Lader, M. Psychological effects of buspirone. J. Clin. Psych. 43: 62–68; 1982.
18. Lister, R. G. The amnesic action of benzodiazepines in man. Neurosci. Biobehav. Rev. 9:87–94; 1985.
19. Lucki, I.; Rickels, K. The behavioral effects of benzodiazepines following long-term use. Psychopharmacol. Bull. 22:424–433; 1986.
20. Lucki, I.; Rickels, K.; Geller, A. M. Chronic use of benzodiazepines and psychomotor and cognitive test performance. Psychopharmacology (Berl.) 88:426–433; 1986.
21. Lucki, I.; Rickels, K.; Giesecke, M. A.; Geller, A. M. Differential effects of the anxiolytic drugs, diazepam and buspirone, on memory function. Br. J. Clin. Pharmacol. 23:207–211; 1987.
22. Mattila, M. J.; Aranko, K.; Seppala, T. Acute effects of buspirone and alcohol on psychomotor skills. J. Clin. Psych. 43:56–61; 1982.
23. McNaughton, N.; James, D. T. D.; Stewart, J.; Gray, J. A.; Valero, I.; Drewnowski, A. Septal driving of hippocampal theta rhythm as a function of frequency in the male rat: Drug effects. Neuroscience 2:1019–1027; 1977.
24. McNaughton, N.; Morris, R. G. M. Chlordiazepoxide, an anxiolytic benzodiazepine, impairs place navigation in rats. Behav. Brain Res. 24:39–46; 1987.
25. McNaughton, N.; Richardson, J.; Gore, C. Hippocampal slow waves generated by reticular stimulation: Common effects of anxiolytic drugs. Neuroscience 19:899–903; 1986.
26. Miyata, H.; Hironaka, N.; Ando, K. Effects of scopolamine and diazepam on delayed discrimination behavior in rats. Jpn. J. Psychopharmacol. 10:343–349; 1990 (in Japanese).
27. Moskowitz, H.; Smiley, A. Effect of chronically administered buspirone and diazepam on driving-related skills performance. J. Clin. Psych. 43:45–55; 1982.
28. O'Keefe, J.; Nadel, L. The hippocampus as a cognitive map. Oxford: Clarendon Press; 1978.
29. Parker, E. S.; Birnbaum, I. M.; Weingartner, H.; Hartley, J. T.; Stillman, R. C.; Wyatt, R. J. Retrograde enhancement of human memory with alcohol. Psychopharmacology (Berl.) 69:219–222; 1980.
30. Patel, J. B.; Ciofalo, V. B.; Iorio, L. C. Benzodiazepines block-

ade of passive avoidance task in mice: A state-dependent phenomenon. Psychopharmacology (Berl.) 61:25–28; 1979.
31. Peterson, R. C.; Ghoneim, M. M. Diazepam and human memory: Influence on acquisition, retrieval, and state-dependent learning. Prog. Neuro-Psychopharmacol. 4:81–89; 1980.
32. Rodrigo, G.; Lusiardo, M. Effects on memory following a single oral dose of diazepam. Psychopharmacology (Berl.) 95:263–276; 1988.
33. Rowan, M. J.; Cullen, W. K.; Moulton, B. Buspirone impairment of performance of passive avoidance and spatial learning tasks in the rat. Psychopharmacology (Berl.) 100:393–398; 1990.
34. Sanger, D. J.; Joly, D. Anxiolytic drugs and the acquisition of conditioned fear in mice. Psychopharmacology (Berl.) 85:284–288; 1985.
35. Tan, S.; Kirk, R. C.; Abraham, W. C.; McNaughton, N. Chlordiazepoxide reduces discriminability but not rate of forgetting in delayed conditional discrimination. Psychopharmacology (Berl.) 101:550–554; 1990.
36. Traber, J.; Davies, M. A.; Dompert, W. U.; Glaser, T.; Schuurman, T.; Seidel, P. R. Brain serotonin receptors as a target for the putative anxiolytic TVQX7821. Brain Res. Bull. 12:741–744; 1984.
37. Viscardi, A. P.; Heise, G. A. Effects of scopolamine on components of delayed response performance in the rat. Pharmacol. Biochem. Behav. 25:633–639; 1986.
38. Wada, T.; Fukuda, N. Pharmacologic profile of a new anxiolytic, DN-2327: Effect of Ro15-1788 and interaction with diazepam in rodents. Psychopharmacology (Berl.) 103:314–322; 1991.
39. Wada, T.; Nakajima, R.; Kurihara, E.; Narumi, S.; Masuoka, Y.; Goto, G.; Saji, Y.; Fukuda, N. Pharmacologic characterization of a novel nonbenzodiazepine selective anxiolytic, DN-2327. Jpn. J. Pharmacol. 49:337–349; 1989.
40. Yamazaki, N.; Kato, K.; Kurihara, E.; Nagaoka, A. Cholinergic drugs reverse AF64A-induced impairment of passive avoidance learning in rats. Psychopharmacology (Berl.) 103:215–222; 1991.