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Effects of Vinburnine on Experimental Models of Learning and Memory Impairments

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DRAGO, F., M. GRASSI, C. VALERIO, F. SPADARO, V. D'AGATA AND N. LAURIA. *Effects of vinburnine on experimental models of learning and memory impairments.* PHARMACOL BIOCHEM BEHAV **37**(1) 53–57, 1990.—Retrograde amnesia can be induced experimentally in mice by injecting them with scopolamine (3 mg/kg, IP) or by inducing seizures with pentylenetetrazol (50 mg/kg, IP), and in rats by subjecting them to hypobaric hypoxia (at a barometric pressure of 300 mmHg for 3 min). We have studied the effects of vinburnine (VNB) in these amnesic states compared to vincamine (VNC) and nicergoline (NCG), in order to assess its activity on drug-induced learning and memory impairments. Vinburnine reduced the disrupting effect of both scopolamine and pentylenetetrazol-induced seizures on the retention of a step-through passive avoidance behavior in mice and on the acquisition of shuttle-box active avoidance behavior in rats. This effect was dose-related up to 20 mg/kg, the peak effect dose after IP administration, and more pronounced than that of VNC and NCG in some tests. These results indicate that VNB influences learning and memory processes disrupted by a pharmacological manipulation. In particular, as scopolamine acts as anticholinergic drug, it is possible that VNB mechanism of action includes also a stimulation of acetylcholine neurotransmission.

Vinburnine Vincamine Nicergoline Amnesia Hypobaric hypoxia

THE occurrence of learning and memory impairments is a common finding in aged animals (8,18) and men (3,12). This has promoted a great interest in drugs which can be effective in reducing the memory disturbances associated with the elderly, and consequently on animal models which can be used in this search (2). For example, administration of the cholinergic receptor antagonist, scopolamine, results in memory impairments (9,10). The same has been found after pentylenetetrazol-induced seizures (19) and hypoxic hypoxia (16,20). In these animal models, nootropic agents have been found to be effective in reducing learning and memory impairments (7, 9, 13, 25). Among these drugs, vincamine (VNC) and nicergoline (NCG) have been found to reverse experimental models of amnesia in animals, namely scopolamine-induced amnesia (15).

In this paper we describe the effects of vinburnine (l-eburnamonine, 16-oxoeburnane, VNB) in some experimental models of learning and memory deficits in mice and rats. Like VNC, this drug is an alkaloid derived from *Vinca minor* and has been shown to prevent the lethal consequences of hypoxic hypoxia in mice (17) and reduce human brain ischemia (23,24). The study has been carried out comparing the effects of VNB to those of two other nootropic drugs, VNC and NCG.

METHOD

Animals

Male rats of the Wistar strain (weighing 160 ± 20 g) and male mice of the CD-1 strain (weighing 20 ± 5 g) were purchased from Morini (Italy) and housed in the animal facilities for seven days prior to beginning with the experimental procedures with food and water available ad lib. The animals were maintained on a 12-hr light/dark cycle (lights on between 8.00 and 20.00 hr) and at a room temperature of 21°C. Each animal was used only once in the experiments.

Drugs

Vinburnine (Chiesi, Italy) and VNC (Sigma, USA) were dissolved in 1% ascorbic acid, NCG (Sigma, USA) in 0.5% tartaric acid, and the solutions were neutralized by 1 N NaOH and diluted to the required concentration by physiological saline. The solutions were administered intraperitoneally (IP) at the doses of 5, 10 and 20 mg/kg. For VNB the dose of 50 mg/kg was also used. Control animals were injected with the vehicle alone.

Scopolamine HBr (Sigma, USA) and pentylenetetrazol (Sig-

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ma, USA) were also dissolved in physiological saline and administered IP at the dose of 3 mg/kg and 50 mg/kg respectively.

Amnesic Manipulations

The mice received an IP injection of scopolamine (15) or pentylenetetrazol (19). Control animals received an IP injection of the vehicle alone.

The rats were exposed to acute hypobaric hypoxia by means of a hypobaric chamber (Chantiers et Ateliers de Bretagne) able to produce a depression of 300 mmHg (barometric pressure). After 3 min in the chamber, the animals were removed and the behavioral test was initiated. Control rats were introduced in the chamber but were not exposed to hypobaric hypoxia (6).

Behavioral Tests

Passive avoidance behavior was studied in a step-through type of passive avoidance paradigm (1). Briefly, the animals were adapted to the apparatus consisting of a large dark compartment equipped with a grid floor and a mesh-covered elevated runway attached to the front center of the dark chamber. Adaptation training was followed by a single trial in which the animals were placed individually on the elevated platform and allowed to enter the dark box. Three such trials were given on the next day with an intertrial interval of 5 min. After the third trial, the animals received a single 2-sec unavoidable scrambled footshock (0.25 mA, AC) immediately after entering the dark compartment. Retention of the response was tested 24 hr after the shock trial. The animals were placed on the elevated runway and the latency to reenter the shock compartment was recorded up to a maximum of 300 sec.

The acquisition of shuttle-box active avoidance behavior was studied in a single session test as described elsewhere (5). Briefly, the rats were trained to avoid the unconditioned stimulus (US) of a scrambled electrical footshock (0.20 mA) delivered through the grid floor. The conditioned stimulus (CS) was a buzzer presented for 3 sec prior to the US. If no escape occurred within 20 sec of CS/US presentation, the shock was terminated. A maximum of 30 conditioning trials were given with a variable intertrial interval averaging 60 sec. The learning criterion was 5 consecutive conditioned avoidance responses (CARs). For those animals that reached the criterion in less than 30 trials, the remaining trials until 30 were considered as CARs. Indexes of avoidance behavior were the total number of CARs and the number of learners.

Experimental Design

The mice were used for the scopolamine or for the pentylenetetrazol experiments. One hr prior to the retention trial of the passive avoidance test a group of animals were injected with scopolamine or physiological saline and thirty min later with VNB, VNC or NCG. The retention trial was then started thirty min later. Another group of mice were injected with pentylenetetrazol one hr prior to the retention trial. This drug induced tonic-clonic seizures 30–60 sec after injection but not the death of the animals. The treatment with VNB, VNC or NCG was made half an hr later. After thirty min the retention trial was started.

Hypobaric hypoxia-induced amnesia was studied in rats. The animals were exposed to this amnesic manipulation and after thirty min were treated with VNB, VNC or NCG. The shuttle-box test and the test for the retention of passive avoidance behavior were carried out thirty min later.

Animals were killed by decapitation at the end of the behavioral procedure. Data were used only from those animals that showed no gross abnormalities on postmortem examination of heart, lungs, stomach and gut, kidneys, brain.

All experiments were performed blind to treatment between 15.00 and 20.00 hr.

TABLE 1

EFFECTS OF ACUTE INTRAPERITONEAL INJECTION OF VINBURNINE (VNB), NICERGOLINE (NCG) OR VINCAMINE (VNC) ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR (LATENCY IN SEC) OF MICE WITH SCOPOLAMINE-INDUCED AMNESIA

Experimental Groups	Latency in Sec
1. Controls (12)	50.0
Scopolamine (30 mg/kg, IP) +	
2. vehicle (18)	6.5*
3. VNB 5 mg/kg IP (12)	12.0*
4. VNB 10 mg/kg IP (12)	21.5†
5. VNB 20 mg/kg IP (12)	52.0‡
6. VNB 50 mg/kg IP (12)	50.0‡
7. VNC 5 mg/kg IP (8)	15.0†
8. VNC 10 mg/kg IP (8)	20.0†
9. VNC 20 mg/kg IP (8)	64.0‡
10. NCG 5 mg/kg IP (8)	10.0*
11. NCG 10 mg/kg IP (8)	18.5†
12. NCG 20 mg/kg IP (8)	57.0‡

Values are expressed as median. The number of animals per each group is indicated in parentheses.
*Significant difference vs. group 1 ($p<0.01$, Mann-Whitney U-test).
†Significant difference vs. group 2 ($p<0.05$, Mann-Whitney U-test).
‡Significant difference vs. group 2 ($p<0.01$, Mann-Whitney U-test).

Statistical Analysis

The data were analysed using the Dunnett's test for multiple comparisons. The frequency data of the shuttle-box experiments were analysed with the Fisher exact *t*-test. Nonparametric data were analysed with the Mann-Whitney U-test. A p level of 0.05 or less was considered as indicative of a significant difference.

RESULTS

The experimental manipulations in this study were followed by amnesia in mice. In the passive avoidance test, amnesia is indicated by the decrease of the latency time to reenter the dark box. Both scopolamine- and pentylenetetrazol-induced seizures were followed by a decrease of this latency time in mice (Tables 1 and 2). Interestingly, scopolamine-induced amnesia appeared to be more pronounced than that following pentylenetetrazol-induced seizures. The amnesic effects of scopolamine in mice were counteracted by the pretreatment with VNB at the doses of 10 and 20 mg/kg, the dose of 5 mg/kg being totally uneffective. The dose of 50 mg/kg, though followed by a reduction in the experimentally induced amnesia, appeared to be as effective as 20 mg/kg (Table 1). In this experiment, VNC and NCG were also effective in preventing experimental amnesia in mice. The pharmacological potency of these drugs was comparable to that of VNB at the dose range used. The antagonistic action of VNB against the amnesia following pentylenetetrazol-induced seizures was also dose-related, but the dose of 50 mg/kg was followed by a reduction of amnesia less pronounced than that obtained after 20 mg/kg (Table 2). The dose-effect range of VNC and NCG was comparable to that of VNB. However, an effect of VNB 5 mg/kg was seen in this

TABLE 2

EFFECTS OF ACUTE INTRAPERITONEAL INJECTION OF VINBURNINE (VNB), NICERGOLINE (NCG) OR VINCAMINE (VNC) ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR (LATENCY IN SEC) OF MICE WITH AMNESIA AFTER PENTYLENETETRAZOL-INDUCED SEIZURES

Experimental Groups	Latency in Sec
1. Controls (12)	46.0
Pentylenetetrazol (5 mg/kg, IP) +	
2. vehicle (18)	10.0*
3. VNB 5 mg/kg IP (12)	18.0†
4. VNB 10 mg/kg IP (12)	29.5†
5. VNB 20 mg/kg IP (12)	52.0‡
6. VNB 50 mg/kg IP (12)	44.0†
7. VNC 5 mg/kg IP (8)	11.0
8. VNC 10 mg/kg IP (8)	22.0†
9. VNC 20 mg/kg IP (8)	59.0†
10. NCG 5 mg/kg IP (8)	14.0
11. NCG 10 mg/kg IP (8)	21.5†
12. NCG 20 mg/kg IP (8)	58.0‡

Values are expressed as median. The number of animals per each group is indicated in parentheses.

*Significant difference vs. group 1 ($p<0.01$, Mann-Whitney U-test).

†Significant difference vs. group 2 ($p<0.05$, Mann-Whitney U-test).

‡Significant difference vs. group 2 ($p<0.01$, Mann-Whitney U-test).

experiment, while the same dose of VNC and NCG was not effective.

In the present experiments, hypobaric hypoxia was followed by a decrease in learning and memory capacity of rats. In the shuttle-box active avoidance test, a decrease in learning capacity is indicated by the decrease in the number of conditioned avoidance responses (CARs) and in the percentage of learners. In fact, hypobaric hypoxia was followed by a decrease in these behavioral parameters and the retention of passive avoidance behavior in rats (Table 3). The treatment with VNB antagonized the behavioral changes induced by hypobaric hypoxia in a dose-dependent manner. The dose-effect range for VNB was similar to that of VNC and NCG. However, an effect was seen in the shuttle-box test after the injection of VNB 5mg/kg, but not following the same dose of VNC and NCG.

DISCUSSION

The present results show that VNB antagonizes learning and memory impairments in different models of experimental amnesias. Our findings are in agreement with those of Linée *et al.* (21) and suggest that VNB is more potent than VNC and NCG only in a minor extent, while in the experiments of Linée *et al.* (21) the difference is larger.

An important question is the possible involvement of acetylcholine neurotransmission in the behavioral effects of VNB, as it reverted the amnesia induced by the anticholinergic agent, scopolamine. This drug is known to impair the retention of passive avoidance behavior when administered prior to the learning trial of the test. This effect is likely to be mediated by central mechanisms as methyl scopolamine, that poorly crosses the blood-brain barrier, does not affect learning and memory processes (14). Also pentylenetetrazol-induced seizures were followed by an impaired retention of passive avoidance behavior in the present experiments. This type of amnesia has been considered state-dependent as it does not prevent memory storage but rather modifies the conditions under which this process takes place (19). For this reason we have exposed the animals to the amnesic procedure only before the retention test and not before the learning trial. Exposure to hypobaric hypoxia was also followed by a decrease in the acquisition and retention of avoidance behavior of rats. This effect seems to be similar to that following the exposure to hypoxic hypoxia (nitrogen hypoxia) in which the number of animals "remembering" varied directly as a function of the chamber's oxygen concentration (9).

The mechanism by which VNB exerts its effects against experimental amnesias is not known. However, the antagonistic action of VNB on scopolamine-induced memory disruption may be explained with a possible influence of this drug on central cholinergic transmission. In fact, cholinergic agonists, such as physostigmine and arecoline, counteract scopolamine-induced amnesia in rodents (11,25). This possible mechanism has been considered for the antagonistic action of VNC and NCG in scopolamine-induced amnesia in rats (15). It should be noted, however, that VNB also affected impairments produced by pentylenetetrazol seizures and hypoxia. For these two models of amnesia an involvement of cholinergic transmission is not known.

Many studies have demonstrated that VNB possesses a cerebral oxygenator activity in that it improves the oxygen supply to the brain by facilitating erythrocyte shape changing, and it increases glucose consumption and mitochondrial cytocrome-oxidase activity without lactate production (4, 22, 27). In this respect, VNB appears to be more potent than VNC, papaverine, trimetazidine and suloctidil (4). Furthermore, VNB increases the number of mice surviving an 80-sec exposure to 100% nitrogen gas (17) and reduces the electroencephalographic changes induced by asphyxic anoxia in curarized rats (26). Other behavioral effects of VNB include a facilitated acquisition and retention of avoidance behaviors and a reduced immobility in a test of forced swim (behavioral "despair") in old rats and gerbils with chronic cerebrovascular insufficiency (Drago *et al.*, in preparation). The pharmacological profile of this drug seems to be similar to that of nootropic agents. Thus, it is possible that the effects of VNB on memory mechanisms are not specific and selective, but may be due to a general improvement of attention or alertness.

The effects of VNB appeared to be dose-related up to 20 mg/kg and no further increase in the dose-effect curve was observed with 50 mg/kg. Interestingly, DeNoble *et al.* (9) found that the general relationship between the dose and the retention latency in the passive avoidance test can be described as an inverted U-shape function of other nootropic agents, such as VNC, vinpocetine, dihydroergotoxine and aniracetam. In particular, the highest dose tested of dihydroergotoxine produced motor incoordination and the decrease in retention latency may reflect the debilitating effect of this substance. Thus, the highest dose of VNB is not more effective than 20 mg/kg because the dose-effect curve for VNB may also be of an inverted U-shape. This has been confirmed by an ancillary experiment where the dose of VNB 100 mg/kg did not exert any effect on passive avoidance retention of rats with amnesia induced by hypobaric hypoxia.

Although its mechanism of action remains to be elucidated, the present results confirm the activity of VNB in protecting animals from the disruption of learning and memory processes induced by different factors. The possibility that VNB may interfere with central cholinergic neurotransmission deserves further studies.

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TABLE 3

EFFECTS OF ACUTE INTRAPERITONEAL INJECTION OF VINBURNINE (VNB), NICERGOLINE (NCG) OR VINCAMINE (VNC) ON THE ACQUISITION OF SHUTTLE-BOX ACTIVE AVOIDANCE BEHAVIOR (CARs AND LEARNERS) AND ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR (LATENCY IN SEC) OF RATS EXPOSED TO HYPOBARIC HYPOXIA

Experimental Groups	CARs	Learners	Latency
1. Controls (10)	12.6 ± 0.5	80	62.0
Hypobaric hypoxia +			
2. vehicle IP (16)	4.6 ± 0.1*	12.5‡	19.5¶
3. VNB 5 mg/kg IP (10)	7.1 ± 0.4†	20	32.0#
4. VNB 10 mg/kg IP (10)	10.0 ± 0.5†	40§	50.0#
5. VNB 20 mg/kg IP (10)	11.0 ± 0.9†	80§	68.0**
6. VNB 50 mg/kg IP (10)	13.7 ± 0.8†	50§	60.5#
7. VNC 5 mg/kg IP (8)	6.2 ± 0.3	12.5	28.7#
8. VNC 10 mg/kg IP (8)	11.0 ± 0.5†	50§	53.5#
9. VNC 20 mg/kg IP (8)	12.5 ± 0.6	75§	59.0#
10. NCG 5 mg/kg IP (8)	8.3 ± 0.5†	12.5	34.5#
11. NCG 10 mg/kg IP (8)	11.1 ± 0.9†	62.5§	56.0#
12. NCG 20 mg/kg IP (8)	13.4 ± 1.1†	75§	54.0#

Values are expressed as mean ± SEM for CARs, percentage for "learners," and median for the retention latency. The number of animals per each group is indicated in parentheses.

*Significant difference vs. group 1 ($p<0.05$, Dunnett's test).
†Significant difference vs. group 2 ($p<0.05$, Dunnett's test).
‡Significant difference vs. group 1 ($p<0.05$, Fisher exact t-test).
§Significant difference vs. group 2 ($p<0.05$, Fisher exact t-test).
¶Significant difference vs. group 1 ($p<0.05$, Mann-Whitney U-test).
#Significant difference vs. group 2 ($p<0.05$, Mann-Whitney U-test).
**Significant difference vs. group 2 ($p<0.01$, Mann-Whitney U-test).

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