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Vinpocetine Enhances Retrieval of a Step-Through Passive Avoidance Response in Rats

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DENOBLE V. J. *Vinpocetine enhances retrieval of a step-through passive avoidance response in rats.* PHARMACOL BIOCHEM BEHAV **26**(1) 183–186, 1987.—Vinpocetine, vincamine, apovincaminic acid, vinconate, aniracetam, Hydergine®, and pemoline were evaluated for their ability to enhance retrieval of a step-through passive avoidance response in rats. The percentage of rats performing the avoidance response was found to decrease as a function of the number of days between training and retention testing (Day 1, 100%; Day 2, 65%; Day 3, 23%; Days 4 and 5, 0%). Vinpocetine administered 60 minutes prior to testing for retention significantly increased the number of rats performing the passive avoidance response. Retrieval enhancement was dose-related in an inverted U-shaped function with the effective doses at 18 and 30 mg/kg PO. In contrast, apovincaminic acid (1–400 mg/kg PO), vincamine (1–200 mg/kg PO), vinconate (1–200 mg/kg PO), aniracetam (1–300 mg/kg PO), Hydergine® (0.1–10 mg/kg PO), and pemoline (1–30 mg/kg PO) were not effective. These data support the view that vinpocetine has cognition-activating abilities as defined in an animal model of memory retrieval.

Vinpocetine Passive avoidance retrieval Memory Rats

VINPOCETINE (3α,16α eburnamenine 14-carboxylic acid ethyl ester), an eburnamenine derivative chemically related to vincamine and vinconate, is active in preclinical tests used to identify cognition activating drugs. Specifically, vinpocetine has protective effects against both scopolamine- and hypoxia-induced impairment of passive avoidance retrieval in rats [6]. More recently, it was reported that the compound increases the acquisition rate of schedule-controlled behavior in rats [7]. Vinpocetine is also known to delay the onset of ischemic-induced convulsions and death in rats [12].

In humans, vinpocetine affects memory recall. For example, vinpocetine improved immediate and delayed word recall in patients with cerebrovascular disorders [10]. In addition, in normal volunteers vinpocetine improved performance on the Sternberg Memory Scanning Task [19]. The latter effect was attributed to vinpocetine's action on central cognitive processes.

The purpose of the present study was to extend the preclinical profile of vinpocetine by determining its effect on memory retrieval in a test of passive avoidance in which retention of the avoidance response decreases with the passage of time.

METHOD

Animals

Male Sprague-Dawley rats (Charles River Breeding Labs, Wilmington, MA) between 90 and 120 days old and weighing 175–225 g at the beginning of the experiment were used. They were housed six per group in 26×36×25 cm stainless steel cages with food and water available. The animals were maintained on a 12-hr light/dark cycle (light on from 0700 to 1900 hr) and at a room temperature of 22–24°C with a relative humidity of 60%. Training and testing for retention was performed between 0800 and 1200 hours.

Apparatus

Experimental sessions were conducted in a two-compartment shuttle box. One compartment, made of clear plastic, measured 17×19×23 cm and was covered by a removable wire mesh grid; the other compartment, made of black arborite, measured 30×20×20 cm and had a floor made of 3 mm stainless steel rods spaced 2 cm apart. The two compartments were separated by a solenoid-operated guillotine door (Lafayette Inst. Co. 85013). On either side of the door a photocell light source and transducer were mounted with the beams parallel to the door opening. A constant current shock generator (Lafayette Inst. Co. 82404-5) was connected to the steel rods in the darkened shock compartment through a shock scrambler. All experimental events were programmed, and responses were recorded by electromechanical and solid state equipment.

Passive Avoidance Training

Passive avoidance training was begun by placing the rat into the clear compartment and, after a 10 sec delay, raising the guillotine door. When the rat moved completely into the darkened compartment, the door was closed, and, following

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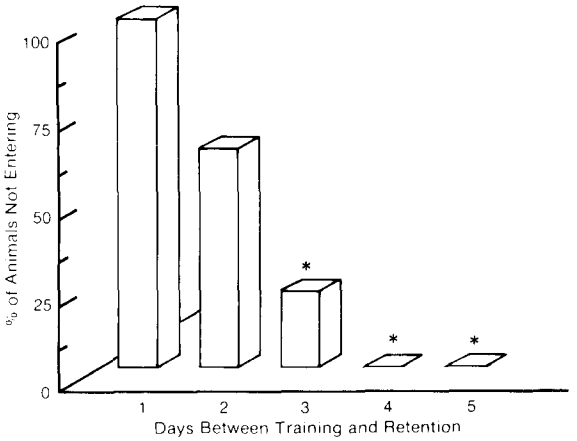

FIG. 1. The percent of animals retaining the passive avoidance response (i.e., not entering the dark compartment within 180 seconds) is shown as a function of the number of days between training and retention testing. Each bar shows the average of 32 vehicle-treated rats (dosed with methyl cellulose) tested in 4 groups of 8, that did not enter the dark chamber. Asterisks above the bars indicate percentages that were significantly different from the Day 1 retention interval (Fisher's Exact Test; $p<0.05$).

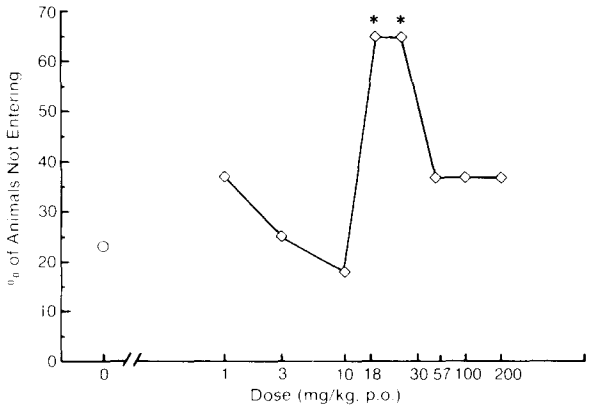

FIG. 2. The percent of animals not entering the dark chamber three days after training is shown as a function of vinpocetine administered 60 minutes before retention testing. The point above 0 represents the average number of rats (N=96, 12 groups × 8 rats each) treated 60 minutes before retention testing with vehicle that did not enter the dark chamber. Each drug point is the mean percent of 16 (tested in two groups of 8) rats that did not enter the dark chamber. Vincamine, apovincaminic acid, vinconate, aniracetam, Hydergine®, and pemoline were not active in this test. Asterisks indicate data points that are significantly different from data obtained from vehicle-treated rats (Fisher's Exact Test; $p<0.05$).

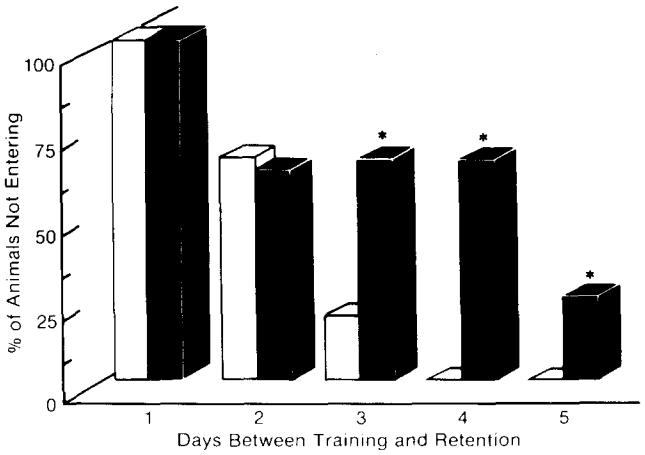

FIG. 3. The effect of vinpocetine on the percent of rats retaining the avoidance response is shown as a function of days between training and retention testing. The open bars show the average of 16 rats (tested in groups of 8) dosed with vehicle. The solid bars show the average of 16 rats, (tested in groups of 8) dosed with the peak effective dose of vinpocetine (30 mg/kg PO) that did not enter the dark chamber. Asterisks above the solid bars indicate percentages that were significantly different from percent of the vehicle-treated rats that did not enter the dark chamber tested at the same respective training retention test intervals (Fishers Exact Test; $p<0.05$).

a 3-sec delay, a shock of 0.2 mA was applied through the grid floor for 2 sec. These footshock parameters were chosen on the basis of a series of pilot experiments indicating that the values were the lowest that could reliably produce 100% retention performance tested one day after training. Immediately after receiving the shock, the rat was removed from the dark compartment and returned to its home cage. A retention test was given one, two, three, four, or five days later. The test proceeded in a manner similar to training, except that the guillotine door did not close if the rat entered the dark compartment, and the shock was not applied to the grid floor. During all retention tests, the rats were provided access to the dark compartment for 180 sec.

Drug Preparation and Administration

Vinpocetine (1–200 mg/kg), vincamine (1–200 mg/kg), apovincaminic acid (1–400 mg/kg), vinconate (1–200 mg/kg, Omni Chemicals), aniracetam (1–300 mg/kg, Hoffmann-La Roche), Hydergine® (0.1–10 mg/kg, Sandoz), and pemoline (1–30 mg/kg, Abbott) were suspended in 0.5% w/v methyl cellulose and administered orally in a volume of 1 ml/kg of body weight 60 min prior to the retention test. All doses are expressed as the base substance, and doses were tested according to a log base 3 sequence. If any dose of a compound significantly increased retrieval, half-log doses above and below the active dose were tested, and active doses were retested in separate groups. Following retesting a separate replication of the effects produced by the peak effective dose at each of the five retention intervals was also determined.

Data Analysis

Using Fisher's Exact Test ($p<0.05$), a comparison was made between treated and vehicle control groups (N=8 per group) with respect to the percentage of animals not entering the dark chamber during the retention test.

RESULTS

The number of rats that retained the passive avoidance response decreased as a function of the number of days between training and testing for retention. One day after training, all rats retained the response, following which the percentage of rats demonstrating retention two, three, four, and five days after training decreased to 62, 25, 0, and 0%, re-

TABLE 1

PERCENT OF RATS SHOWING RETRIEVAL AFTER DOSING WITH VEHICLE OR TEST COMPOUNDS THREE DAYS AFTER AVOIDANCE TRAINING

	Dose (mg/kg PO)*										
Drug	0†	0.1	0.3	1	3	10	30	100	200	300	400
	Percent										
Vincamine	25	—	—	25	0	12	40	28	0	—	—
AVA	12	—	—	0	25	18	0	0	0	0	0
Vinconate	20	—	—	0	16	18	0	25	0	—	—
Aniracetam	28	—	—	37	40	25	25	25	40	0	—
Pemoline	30	—	—	0	0	25	12	—	—	—	—
Hydergine®	30	0	25	28	12	0	—	—	—	—	—

*Each dose was tested in a minimum of 8 rats.
†Vehicle control (methyl cellulose).
—Not tested.
AVA=Apovincaminic Acid.

spectively (Fig. 1). These data were obtained from vehicle-treated rats.

A dose-response curve was obtained for vinpocetine, and at a dose of 18 and 30 mg/kg PO vinpocetine enhanced the three-day, time-induced retention deficit (Fig. 2). Doses below 18 mg/kg PO and above 30 mg/kg PO were ineffective in increasing the percentage of rats exhibiting retrieval of the passive avoidance response. The number of rats retaining the avoidance response first increased, then decreased as the vinpocetine dose was increased, producing an inverted U-shaped dose response curve.

In separate groups of rats the peak effective dose of vinpocetine (30 mg/kg PO) was tested at five retention intervals (i.e., one, two, three, four, and five days after training). Vinpocetine significantly increased the number of rats that retained the avoidance response when tested on Days 3, 4, and 5 (Fig. 3). The number of rats demonstrating the avoidance response on Days 1 and 2 was not increased by vinpocetine.

Apovincaminic acid (1–400 mg/kg), the major metabolite of vinpocetine, vincamine (1–200 mg/kg), vinconate (1–200 mg/kg), aniracetam (1–300 mg/kg), Hydergine® (0.1–10 mg/kg), and pemoline (1–30 mg/kg) were not effective in significantly increasing the number of rats performing the passive avoidance response three days after training (Table 1).

DISCUSSION

The number of rats demonstrating retrieval of a passive avoidance response decreased as a function of the time between training and retention testing. While many reports document that retention of a passive avoidance response can be disrupted by both pharmacologic and physiologic insults, e.g., treatment with scopolamine [6] or exposure to hypoxia or electroconvulsive shock [5, 6, 13], there have been few reports in which the retrieval of a single-trial passive avoidance response decays with time [2,9]. The present results are in general agreement with those of Gold *et al.* [9], who reported that retrieval of an inhibitory avoidance response in rats decreased as a function of age and the time between training and retention testing.

The decrease in the number of rats that stayed out of the dark chamber when the number of days between training and testing for retention was increased can be attributed to both the training-interval and to the low shock level (0.2 mA). In most studies of passive avoidance, grid shock intensities range from 0.5 to 1.5 mA, and retention intervals rarely extend beyond one day [1]. The findings that most rats entered the dark chamber during the retention test on Day 3 and that all rats entered on Days 4 and 5 indicates that low shock level is critical to this model. The results indicate that under the present experimental conditions, the decrease in retention may result from a naturally occurring memory decay.

Of the compounds tested, only vinpocetine at doses of 18 and 30 mg/kg PO administered prior to the retention test three days after training significantly increased retrieval. Using the peak effective dose (i.e., 30 mg/kg PO) vinpocetine also increased retrieval of the passive avoidance response on Days 4 and 5. The latter observation is particularly interesting since vehicle-treated rats showed no evidence of retrieval on Days 4 and 5. The dose-response relationship is a narrow, inverted U-shaped curve. Although the reason is not known, this dose-response relationship is frequently obtained in studies with other compounds that reportedly improve memory [3, 5–7, 17]. It was also demonstrated that vinpocetine, and not its major metabolite apovincaminic acid, is the active substance.

Compounds active in preventing scopolamine- or hypoxia-induced impairment of passive avoidance [5,6] were inactive in this retention test. Neither vincamine nor vinconate, compounds chemically similar to vinpocetine, nor aniracetam, Hydergine®, or pemoline facilitated memory recall in rats. The increase in the number of rats dosed with vinpocetine that did not enter the dark chamber cannot be explained by a supression of motor function. An evoked motor response in mice is not altered by up to 100 mg/kg PO of vinpocetine [11]. Moreover, if the enhanced retrieval was a result of motor impairment, higher doses of vinpocetine (i.e., >30 mg/kg) used in the present study would have also increased the number of rats not entering the dark chamber. This was not found, and rats dosed with up to 200 mg/kg of vinpocetine entered the dark chamber (Fig. 2). In addition, an ancillary experiment in which rats were dosed with vinpocetine (30, 100 and 200 mg/kg PO) before training showed that entry latencies were not different from rats dosed with vehicle (30 sec ±5 SEM, 28 sec ±3 SEM, respectively). These data suggest a pharmacologic activity for vinpocetine in memory recall without alterations in motor function.

The mechanism(s) by which vinpocetine enhances retrieval is as yet unknown. However, vinpocetine reportedly increases the turnover of norepinephrine in brain [4,13], and central monoamine transmitter systems are known to have modulating effects on selective attention [16,18] and memory retrieval [14, 17, 18]. Therefore, the compound's action on ascending noradrenergic pathways should be considered. The present data suggest that vinpocetine, a compound which increases memory recall in human patients and volunteers, also promotes memory retrieval in a passive avoidance paradigm in rats.

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