Pharmacology Biochemistry and Behavior, Vol. 46, pp. 415–421, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of Idebenone on Information Processing in Aged Long–Evans Rats

MARY ANN PELLEYMOUNTER[1,2] AND MARY JANE CULLEN[1]

Program in Neuroscience, Oberlin College, Oberlin, OH 44074

Received 8 October 1992

PELLEYMOUNTER, M. A. AND M. J. CULLEN. *Effects of idebenone on information processing in aged Long–Evans rats.* PHARMACOL BIOCHEM BEHAV **46**(2) 415–421, 1993. – Idebenone (6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone) is a benzoquinone that has been shown to improve cognitive function in animals subjected to cerebral ischemia and in rats with lesions of the basal forebrain cholinergic system. Because the cognitive deficits observed in aged rats have been associated with decreased cerebral blood flow and basal forebrain cholinergic dysfunction, it was hypothesized that IDE might improve cognition in aged animals. In the present study, the effects of idebenone on cognitive function in aged Long-Evans rats were assessed using a battery of tests that evaluated attention, habituation, and spatial learning. Selective attention was assessed using an overshadowing paradigm, where IDE (30 mg/kg, IP) was injected 30 min prior to compound cue exposure. IDE enhanced the overshadowing effect in aged rats. The Morris water maze was used to assess spatial learning, where IDE (3 mg/kg, IP) was injected daily throughout the course of training. IDE did not improve the impaired performance of aged rats in the Morris task. Habituation was tested by measuring recovery from gustatory neophobia. IDE (30 mg/kg, IP) was injected 30 min prior to the first exposure to the novel taste. IDE normalized habituation rate in aged rats. It was concluded that IDE improves some forms of acquisition in aged rats, and may do so by decreasing general reactivity to novel stimuli.

Idebenone Aging Rat Cognition Attention Reactivity Spatial learning

IDEBENONE [6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone; IDE] is a quinone derivative that can act as a neuroprotective agent against ischemic insult in animals (35). There is evidence, for example, that idebenone can reduce damage in tissue exposed to excitotoxins (28) and in hypoglycemic tissue (20). In addition, IDE can reverse cognitive deficits that result from ischemic insult. Impairments in passive avoidance retention, working memory, and delayed alternation can be reduced or reversed by pretraining administration of IDE at doses ranging from 3–30 mg/kg (18,19, 34,45). Deficits in working memory and in passive avoidance retention in rats with nucleus basalis lesions can also be reduced with IDE administration (29,45).

Interestingly, aged rats demonstrate cognitive deficits that are similar to those observed in ischemic animals and in rats with nucleus basalis lesions. There is a plethora of data demonstrating working memory deficits in aged rats (1,13,43). Deficits in passive avoidance retention (6,9,24,25,38,46), delayed alternation (43), spatial memory (7,8,37), and complex maze learning tasks have also been observed in aged rats (14,15).

The decline in cognitive function that has been observed in some aged rats has been associated with a variety of neurobiological changes, including decreased cerebral blood flow (11, 12,22,41), decreased brain oxidative metabolism (10,21), and decreased regulatory capacity of hippocampal and cortical cholinergic systems [for review, see (5,15,38)]. It is possible, then, that aged rats showing cognitive decline might benefit from quinone derivatives like IDE, which act to increase cerebral blood flow, enhance brain oxidative metabolism (32, 33,35), and reduce or prevent the decline in cholinergic function that accompanies cerebral ischemia or nBM lesions (16,17).

In the following experiments, we tested the effects of IDE on several forms of information processing in the same rats, including selective attention, neophobia, habituation, and spatial learning.

METHOD

Subjects

Subjects in these experiments were 38 male Long-Evans hooded rats. All rats were individually housed in a vivarium with a 12 L : 12 D cycle, where ambient temperature was held constant at 25°C. Animals received food (Purina Lab Chow) and water ad lib unless they were being trained for the selective

[1] Current address: Department of Neurobiology (5-1-A-226), Amgen, Inc., Amgen Center 1840 DeHavilland Drive, Thousand Oaks, CA 91320.
[2] To whom requests for reprints should be addressed.

attention task or the neophobia habituation task. Aged rats (n = 18) were retired breeders obtained from Charles River Labs (Wilmington, DE) at 8 months of age. These rats were 26 months old at the time testing began. Young counterparts (also obtained from Charles River) were 6 months of age. Testing was completed for each animal 2 months later, when aged and young rats were 28 and 8 months old, respectively.

The test battery was administered in the following order: a) selective attention (overshadowing of tone over light); b) spatial learning (Morris water maze); c) general attention and simple memory (taste neophobia and habituation). The test battery was administered to all rats in the same order because of the inevitable attrition due to normal aging and because of small group numbers in the original population of old rats. Rats were always given 2 drug-free weeks between experiments. This should have been sufficient for clearance of the drug from the periphery because pharmacokinetic characterization of IDE dosing in rats has indicated that complete clearance of the drug from plasma is accomplished in 48 h (personal communication, TAP Pharmaceuticals).

Drug Administration

IDE (Tap Pharmaceuticals, Deerfield, IL) was administered in a 0.9% saline, 5% gum arabic vehicle (VEH) that was sonicated to produce a fine suspension which was injected IP in all experiments. IDE was administered at a dose of 30 mg/kg for all tests where it was injected on an acute basis; previous work with the effects of this compound on passive avoidance retention had suggested that this was an optimal dose (34). In experiments where a chronic injection schedule was required, however, IDE was administered at 3 mg/kg to avoid any cumulative adverse effects, particularly on aged rats. The 3-mg/kg dose reduced amnesia produced by cerebral ischemia or nucleus basalis lesions in passive avoidance paradigms that involved multiple dosing (19,34). A complete dose–response study would have been impossible because of limitations inherent with aged animal research, including smaller ns and attrition.

Procedures

Selective attention. Selective attention was assessed using an overshadowing paradigm. Overshadowing could be characterized as testing a particular form of selective attention, where an animal attends to the stronger of two stimuli that have been simultaneously paired with footshock. It, like most tests of selective attention, involves parameters other than attention. Overshadowing, for example, requires associative learning and the ability to distinguish sensory stimuli of different intensities, along with the ability to react selectively to these sensory stimuli. In the overshadowing paradigm, a compound cue consisting of one intense and one weak stimulus was paired with shock or a reward. Previous work has shown that the normal subject responds to the intense cue as if it predicted outcome but responds weakly, at best, to the less intense stimulus. This "overshadowing" of the weak stimulus by the intense stimulus has been attributed to the idea that the subject forms a much stronger association between the intense cue and outcome than between the weak cue and outcome because the subject failed to *attend* to the weak cue when it was presented along with the intense cue (23,26).

Procedure. All subjects were water deprived for 3 days, in that they were given water every 24 h for 30 min. On the fourth day, subjects were placed into Coulbourn test cages (10 × 12 × 13 in.) that were housed inside a Coulbourn (E-10-20) isolation chamber for the first 15 min of the 30-min drinking interval. The last half of the drinking interval was conducted in the animals' home cage throughout training; this was done so that animals would be equally thirsty from day to day. The water spout in the test cages was placed between two photocells in such a way that licks could be monitored during the entire drinking interval. Baseline drinking was assessed for 3 days using the Coulbourn cages (days 4–6). On day 7, rats were injected with either IDE (30 mg/kg, IP) or VEH 30 min prior to being placed into the test cage. Nine aged and 10 young rats received IDE; similarly, 9 aged and 10 young rats received VEH. Rats were then given three overshadowing training trials, where a 92-dB tone was presented along with a dim amber LED cue light (Coulbourn E11-02) for 30 s. The cue light was 10.16 cm from the grid floor and 3.16 cm from the lick spout. The tone was presented through a 2 × 2-in. speaker that was positioned 25.4 cm from the grid floor, directly above the cue light. Immediately following presentation of the compound cue, rats were subjected to a 0.4-mA, 1-s scrambled footshock, completing the trial. Intertrial interval length was pseudorandom, with a mean of 60 s and a range of 50–70 s. Licks were measured throughout the 15-min session. On day 8, rats were given three similar trials with the cue light alone. On day 9, rats were tested with the tone alone. The response assessed was lick suppression because a common response to anticipation of footshock is to "freeze," which results in a suppression of licking behavior. Lick suppression on both test days was calculated using the ratio: no. of licks during the first cue presentation/no. of licks during first cue presentation + no. of licks during the last 30 s of the preceding intertrial interval.

Spatial learning in the Morris water maze. The Morris water maze has been well characterized as a measure of spatial learning and memory (30,31,42). The basis for the task is that a rat must locate a hidden escape platform based upon its relative position to surrounding objects, or cues. The escape platform is "hidden" by submerging it 1 cm below the surface of opacified water in a large pool. It has been hypothesized that rats learn to locate the platform by forming and storing a "cognitive map" of the maze environment (30,36,42).

Procedure. Acquisition of spatial learning was assessed in a 6-ft. diameter, 2-ft. deep water maze. The maze was surrounded by white curtains. Four high-contrast pictures were attached to the inside face of these curtains in a random fashion, hanging 6 in. above the rim of the tank. Pictures varied in size, ranging from 24 × 12 to 30 × 24 in. The maze contained tepid water (28°C) that was opacified with powdered milk (900 g). The white, 10-cm diameter escape platform was submerged 1 cm below the surface of the water. This escape platform was stationary relative to the position of the pictures throughout training. A closed-circuit TV camera was centered above the maze. This camera allowed us to videotape rats during probe trials so that quadrant time and annulus crossings could be calculated during probe trials for each rat.

Rats were placed into the maze at one of the cardinal start points (NSEW) and the latency to locate the hidden platform was measured. If the rat did not locate the platform within 120 s, it was placed onto the platform by the experimenter. Upon finding the platform, the rat was allowed to remain on the platform for 30 s and was then allowed to rest in a dry "holding" cage for 60 s. The rat was then put into the maze for the next trial at a different start point. Rats were given three trials each day, and were injected with either IDE at a dose of 3 mg/kg (n = 9 aged and 9 young) or VEH (n = 9 aged and 10 young) 30 min prior to the beginning of each daily session.

Every sixth trial, the escape platform was removed and

the rat's swim path videotaped for 30 s. These "probe" trials provided an index of spatial bias, using measures such as quadrant time and annulus crossings. Quadrant time is based upon the idea that the maze can be divided into four equal quadrants, and the amount of time swimming in the quadrant that formerly contained the escape platform should be greater than time spent in any other quadrant. Our quadrant time criteria was 10 s. An annulus crossing is a swim over the exact former position of the escape platform. We used two crossings for our criteria. Previous work has shown that animals that meet these criteria show significant spatial bias (37). All rats were trained on the water maze until they met our spatial criteria.

Taste neophobia and recovery. Rats are normally neophobic to novel tastes, cues, and environments (2,4,27). Gustatory neophobia is a protective species-specific behavior in rats and can be observed as decreased ingestion of the novel substance (in comparison to ingestion of a familiar substance). Gustatory neophobia can be decreased by dorsal noradrenergic bundle lesions (4), suggesting that it could reflect a general form of attention, such as arousal. Recovery from gustatory neophobia is also a commonly observed phenomena and could be viewed as a measure of simple, nonassociative learning such as habituation (2,3,27).

Procedure. Rats were maintained on the water deprivation schedule that had been used for the overshadowing study. Baseline drinking was measured in two 15-min intervals on the day immediately following completion of the overshadowing study, and was continued for 5 days. All measurements were taken away from the subjects' home cages. Instead, they were taken in 18 × 9 × 6-in. Plexiglas cages in the same room every day. On day 6, rats were injected with either IDE (30 mg/kg, IP) or VEH. Six aged and seven young rats were injected with IDE; similarly, seven aged and seven young rats were injected with VEH. (These smaller *n*s reflected attrition.) Thirty minutes after injection, they were presented with grape juice (1 can Welch's grape juice in 2 qt water) for the first 15 min of the 30-min interval and then allowed to drink water for the last 15 min of the interval. Both grape juice and water intake were measured. Rats were given the same regimen of grape juice followed by water for the next 3 days but were not injected. Ratios of grape juice consumed/water consumed were calculated for each day.

RESULTS

Selective Attention as Assessed by Overshadowing

An overall analysis of variance (ANOVA) comparing the factors age, drug, and condition for lick suppression revealed that there was no age effect; in other words, aged rats showed as much overshadowing of the light by the more intense tone as did their young counterparts. There was, however, a significant overall effect of both drug, $F(1, 52) = 7.85$, $p < 0.007$, and condition, $F(1, 52) = 6.82$, $p < 0.012$. The significant condition effect suggested that all rats showed overshadowing of tone over light, although posthoc analysis revealed that aged rats suppressed significantly more than young rats to both tone and light ($p < 0.001$). Post hoc analysis of the drug effect suggested that its statistical significance was due to the large effect of IDE on aged rats ($p < 0.001$). Overshadowing in young rats was not significantly affected by IDE. There was also a significant interaction between age and drug, $F(1, 52) = 9.64$, $p < 0.003$. This was due to the fact that aged rats treated with IDE showed more overshadowing than any other group ($p < 0.05$). All of these results can be observed in Fig. 1.

Spatial Learning as Assessed by Acquisition of the Morris Maze Task

An ANOVA that compared the factors of age, drug, and trial number using latency to reach the escape platform as the

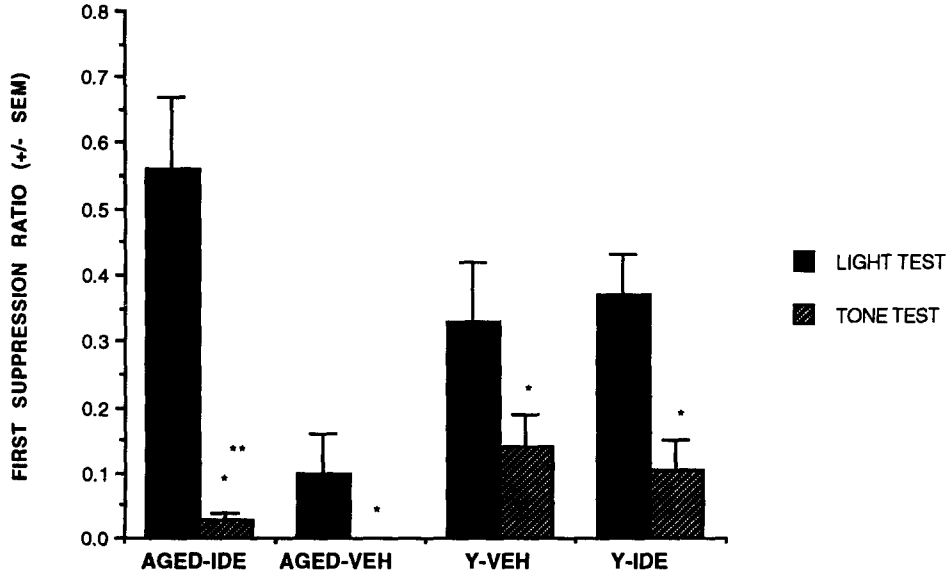

FIG. 1. Overshadowing of light by tone in young and aged Long Evans rats. This illustration compares the suppression ratios during the light test and the tone test for AGED-idebenone (IDE) ($n = 9$), AGED-vehicle (VEH) ($n = 9$), young (Y)-IDE ($n = 10$), and Y-VEH-treated rats ($n = 10$). Larger suppression ratios indicate less association of the stimulus with shock and suggest that less attention was paid to that stimulus. *$p < 0.01$, tone vs. light; **$p < 0.05$, aged vs. young.

dependent variable revealed that there was a significant effect of age, $F(1, 32) = 9.4$, $p < 0.0044$, and trial number, $F(17, 576) = 26.3$, $p < 0.001$. Posthoc analyses showed that all rats required less time to reach the escape platform as trial number increased, indicating that all rats were learning the task ($p < 0.05$; data not shown). A separate ANOVA that compared the factors of age and drug for number of trials to reach spatial bias criterion showed that there was a significant effect of age, $F(1, 34) = 9.8$, $p < 0.004$. Post hoc analysis revealed that aged, VEH-treated rats required significantly more probe trials to reach criterion than did young, VEH-treated or young, IDE-treated counterparts ($p < 0.01$). There were no significant effects of IDE in either age group (see Fig. 2).

General Attention and Nonassociative Learning as Assessed by Gustatory Neophobia and Recovery

ANOVA comparing age and drug over the 4 days of grape juice exposure revealed an overall effect of age, $F(1, 23) = 6.53$, $p < 0.018$, and day, $F(3, 69) = 48.28$, $p < 0.001$. There was also a significant drug × day interaction, $F(3, 69) = 2.6$, $p < 0.058$. Figure 3 illustrates a trend for the aged IDE group to suppress grape juice intake more than other groups on the first and second days of exposure. However, on the third day of exposure the grape juice/water ratio for the aged IDE group was similar to that of young animals; in other words, they had habituated to the novel flavor to the same extent as young rats. The aged vehicle group, in contrast, was still suppressing grape juice intake ($p < 0.048$) on the third day of exposure. On the fourth day of exposure, significant age differences had disappeared, although aged vehicle rats were still suppressing more than any other group. There were no IDE differences in the young group. The only significant difference for young animals was an increased grape juice/water ratio from day to day ($p < 0.001$), indicating initial neophobia followed by habituation. Small groups of rats treated with or without IDE from both age groups were also given only water to drink throughout the study. Neither IDE nor age significantly affected drinking or the ratio of first/last 15-min water consumed.

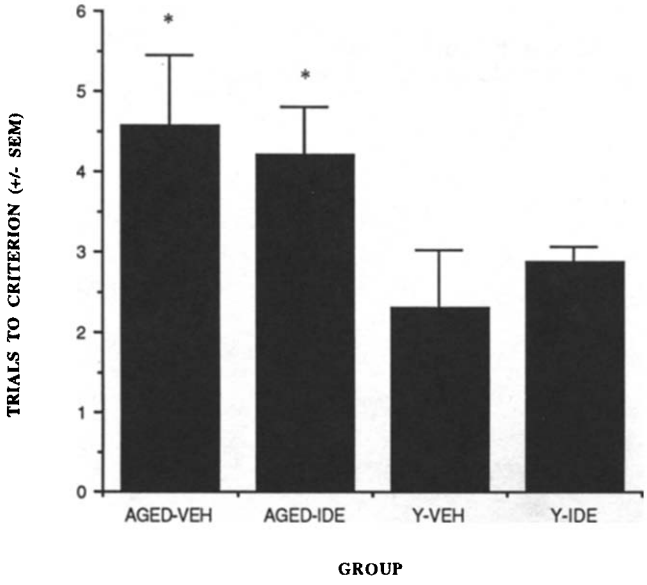

FIG. 2. Trials to criterion for the Morris water maze in young and aged rats. This graph illustrates the mean number of trials to criterion in young and aged rats treated with either idebenone (IDE) or vehicle (VEH) (3 mg/kg/day). Subjects could meet criterion during probe trials if they spent at least 10 of 30 s in the training quadrant and crossed the training annulus at least twice during the 30-s trial. Aged rats required a significantly greater number of trials to meet this criterion than did their young counterparts (*$p < 0.01$). IDE did not significantly affect the number of trials to criterion. The number of animals/group was the same as in Fig. 1 except that there were only nine young (Y)-IDE rats.

DISCUSSION

Aged rats were slower to recover from taste neophobia and required more training trials to reach criterion performance in the Morris water maze. In contrast, these aged animals showed a stronger overshadowing effect than young counterparts. IDE exaggerated the overshadowing of tone over light in aged rats and significantly improved the performance of these aged animals in recovery from taste neophobia. IDE, however, did not improve learning in the Morris water maze.

Overshadowing

Aged rats appeared to be more sensitive to the intensity differences of neutral stimuli than did their young counterparts in the overshadowing task, which was reflected in the large overshadowing effect seen in these aged rats. Further, aged rats suppressed to both tone and light more than their young counterparts. IDE, however, reversed the suppression to light in aged rats without affecting the exaggerated suppression to tone in these animals. As suggested in the Method section, overshadowing has been viewed as a test of selective attention. However, it can also be viewed as a test of associative learning or a test of reactivity to stimuli of differing intensities. If the overshadowing phenomena is viewed as a selective attention paradigm, then these data suggest that aged rats are more capable of selective attention than young animals and that IDE may enhance selective attention in aged rats.

If overshadowing is viewed as a special case of classical conditioning where one conditioned stimulus (CS)–unconditioned stimulus (US) association is simply stronger than another, then our data suggest that aged animals form differential CS–US associations to a greater degree than do young animals and that IDE enhances this differential CS–US association. This idea is in general agreement with earlier data that suggested that aged rats show deficits in classical conditioning *only* if delays are inserted between CS offset and US onset (39,44). If aged rats treated with IDE showed greater overshadowing because they formed stronger CS–US associations, then IDE-treated aged rats would have shown a significantly greater suppression to tone than VEH-treated counterparts, indicating that it strengthened the tone/shock association. Instead, IDE appeared to enhance overshadowing in aged rats by weakening the light/shock association.

It is possible that IDE decreased suppression to light in aged rats because it altered visual perception of the dim light. Data from a group trained and tested on light alone would have answered this question, but the small numbers of aged rats precluded the addition of such a group. This explanation does seem doubtful, though, for several reasons: a) IDE had no effect on the light/shock association in a group of young animals tested and trained on light alone (data not shown); b) if anything, aged rats seemed to notice the light cue more than their young counterparts, as indicated by the large amount of lick suppression during the light test.

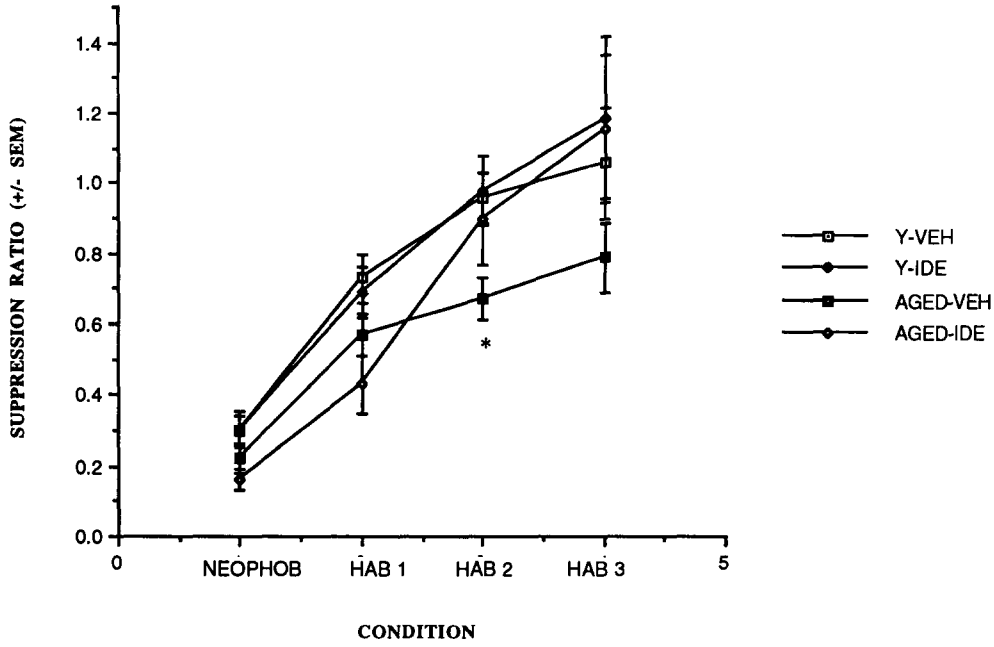

FIG. 3. Neophobia and habituation in young and aged rats. This figure compares suppression ratios (grape/water) for AGED-idebenone (IDE) (n = 6), AGED-vehicle (VEH) (n = 7), young (Y)-IDE (n = 7), and Y-VEH (n = 7)-treated rats. All rats showed significant suppression to their first taste of grape juice on the NEOPHOB day. By the second day of habituation, only AGED-VEH rats were still suppressing significantly more than any other group. $^{**}p < 0.048$, AGED-VEH vs.all other groups.

The most probable explanation for the effects of IDE on overshadowing in aged rats is that aged Long-Evans rats are simply more reactive in general (as indicated by the large amount of suppression to light in the aged-VEH group), and IDE merely acts to decrease general reactivity to the point that an animal can selectively attend to a meaningful stimulus.

Spatial Learning in the Morris Maze

In general, aged rats were impaired on acquisition of the Morris water maze, in terms of escape latencies and spatial bias during the probe trials, which was reflected in the number of probe trials to reach our spatial bias criterion. A similar age-related deficit in acquisition of the Morris task has been demonstrated previously (7,8,37). IDE, however, did not significantly decrease either the number of probe trials to criterion or the escape latencies for aged rats. This might suggest, then, that IDE has no effect on the storage or processing of spatial information in aged rats. [The Morris water maze task has been characterized as a learning paradigm that requires storage and processing of specifically spatial information (30,31)]. The fact that IDE did not improve learning in a task that required storage of specific information is interesting in view of the fact that the Morris task, unlike the gustatory neophobia or overshadowing task, does not appear to be susceptible to reactivity parameters. Reactivity may not have as profound an effect in the Morris maze because it has different response requirements than either overshadowing or habituation to gustatory neophobia.

Neophobia and Recovery

Although all animals showed a similar neophobic response to the novel taste of dilute grape juice, aged VEH-treated rats showed less habituation to the novel taste than young rats, even on the fourth day of exposure. In contrast, aged IDE-treated rats could not be distinguished from young rats by the third day of grape juice exposure. These results might suggest that aged rats do not retain information about the learned "safety" of grape juice as efficiently as their young counterparts and that IDE treatment improves the retention of such information in aged rats.

An alternative explanation for the decreased habituation rate in aged rats could be that aged rats simply require a longer period of time to recover from the emotional response evoked by a novel taste than young rats and IDE treatment might act to diminish long-term physiological effects of the fear evoked by a novel taste. There is evidence that habituation rate in aged rodents is more susceptible to changes in the arousal-inducing properties of the initial stimulus than it is in young rodents (3,40). This second explanation of IDE's effects on learning in aged rats could also be used to understand its effects in the overshadowing task, where IDE appeared to decrease reactivity to the weaker component of the compound CS, dim light. Therefore, these data could suggest that IDE might affect learning and memory in aged animals by decreasing reactivity.

In summary, IDE improved the performance of aged rats in two tasks that have large reactivity/arousal components: overshadowing of tone over light and habituation of a gustatory neophobic response. IDE did not improve performance in a task with a large storage/memory component: place learning in the Morris maze. It is possible, then, to attribute the effects of IDE on cognitive performance in aged animals to reactivity/arousal mechanisms rather than information storage mechanisms. For example, overshadowing was enhanced by decreasing reactivity to the dim light rather than by increas-

ing reactivity to the tone. A similar interpretation could be applied to the habituation to neophobia data. IDE might have increased habituation rate by decreasing the arousal value of the novel taste. Further, IDE did not improve spatial learning in the Morris maze, which is the only task included in the profile that requires long-term storage of several different types of information and may not be susceptible to reactivity. Obviously, there are alternative interpretations for these data; a more direct test that IDE acted to reverse reactivity to novel stimuli in aged rats would be necessary to confirm such a hypothesis.

ACKNOWLEDGEMENT

This work was supported by a drug development contract from TAP Pharmaceuticals, Deerfield, IL 60015

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