British Journal of Pharmacology (1997) 122, 257–264

Effects of VA-045 on learning and memory deficits in traumatic brain injury (TBI)-induced retrograde and anterograde amnesic mice

Ya-Ping Tang, Yukihiro Noda, Takaaki Hasegawa & [1]Toshitaka Nabeshima

Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466, Japan

1 No specific regimen has been developed to treat post-traumatic amnesia in man. In the present study, we examined the effects of (+)-eburnamenine-14-carboxylic acid (2-nitroxyethyl) ester (VA-045), a novel derivative of apovincaminic acid, on learning and memory deficits associated with a mild traumatic brain injury (TBI) in mice.

2 Two kinds of amnesia, TBI-induced retrograde amnesia (TRA) and anterograde amnesia (TAA), were produced by means of post- and pre-acquisition head injury, respectively, by a simple weight-drop device. A novel procedure of water-finding task was used to assess learning and memory functions.

3 Both TRA and TAA mice were dramatically impaired in the task performance, with prolonged latencies for finding and drinking in either retention test or retest, indicating that retention was impaired in TRA mice while learning and retention were impaired in TAA mice.

4 VA-045 administered 30 min post-trauma in TRA mice dramatically shortened the prolonged latencies for finding and drinking in both retention test and retest, indicating that VA-045 significantly improved the retention deficit observed in TRA mice.

5 VA-045 administered 30 min post-trauma in TAA mice dramatically attenuated the prolonged latencies for finding and drinking in both retention test and retest, indicating that VA-045 significantly improved the learning and retention deficits observed in TAA mice.

6 Administration of VA-045 30 min pre-trauma in normal mice markedly attenuated the delay of latencies for finding and drinking after trauma in both retention test and retest, which shows that VA-045 significantly prevented learning and retention deficits after TBI.

7 Motor activities were not significantly affected by either the TBI or the chemical treatment at the time of task examination in either experimental model.

8 It is concluded that VA-045 may have potential effects on learning and memory deficits observed in either TBI-induced retrograde or anterograde amnesia.

Keywords: Traumatic brain injury (TBI); learning; retention; anterograde amnesia; retrograde amnesia; VA-045

Introduction

Cognitive deficits, especially impairments in learning and memory, are the major manifestations of the neurologic sequelae following mild to moderate traumatic brain injury (TBI) in man (Binder, 1986; Levin *et al*., 1987; Levin, 1990; Rutherford *et al*., 1977). Although the majority of patients recover within weeks, some have such severe and prolonged deficits in learning and memory that they require months to years to recover from these disabilities (Russell, 1935; Binder, 1986; Levin *et al*., 1987; Levin, 1990). Any attempts to treat these problems with pharmacological agents have met with little success.

Terms 'retrograde amnesia' and 'anterograde amnesia' have been used broadly to describe learning and memory deficits observed in human TBI (Russell, 1935; Rimel *et al*., 1982; Squire & Cohen, 1984). Retrograde amnesia is defined as a memory loss for events occurring before the onset of injury, and the memory deficits involved in this type of amnesia may be linked to a retention process because acquired information is weakened or disrupted at the time of injury (Rutherford *et al*., 1977; Squire & Cohen, 1984). Anterograde amnesia is defined as memory deficits following injury and characterized by profound deficits in the ability to acquire new information, or in retention after a short delay, despite normal intellectual capacity (Rimel *et al*., 1982; Squire & Cohen, 1984). Analytical approaches to evaluate these kinds of amnesia in the laboratory are available to focus on experimental processes (Gabriel, 1985; Squire, 1986; Gorman *et al*., 1993). That is to say, the animal whose head injury is produced after acquisition training is defined as retrograde amnesia model, while before acquisition training as an anterograde amnesia model.

By using a simple weight-drop device, a mild TBI mouse model has been recently developed in our laboratory (Tang *et al*., 1997a,b). The observations of transient behavioural suppression and long-lasting memory deficits in this model are similar to the reversible loss of consciousness and persistent cognitive deficits exhibited in human concussive brain injury. The technique is simple, readily reproducible and provides some advantages, such as animal cost, sample size, etc. in the pharmacological studies of human mild to moderate TBI in the laboratory. (+)-Eburnamenine-14-carboxylic acid (2-nitroxyethyl) ester (VA-045) is a new compound to which a nitrooxy moiety is introduced at position 14 of apovincaminic acid (Kawashima *et al*., 1993). VA-045 has been shown to enter the central nervous system by crossing the blood-brain-barrier, selectively dilate canine cerebral arteries, increase the cerebral blood flow and improve memory deficits observed in aged animals (Okuyama *et al*., 1994) or associated with disturbance in cholinergic activities (Ogawa *et al*., 1992). However, effects of VA-045 on TBI-induced learning and memory deficits have not been assessed. In the present study, we produced two kinds of amnesia, TBI-induced retrograde amnesia (TRA) and anterograde amnesia (TAA), by means of post- and pre-acquisition head injury, respectively, and then examined effects of VA-045 on retention deficit observed in TRA mice and learning and retention deficits observed in TAA mice, by use of a novel procedure of water-finding latent learning paradigm.

[1] Author for correspondence.

Methods

Animals and drugs

Male ddY mice (Nihon SLC, Shizuoka, Japan) weighing between 30 and 35 g at the beginning of the experiments were used. The animals were housed in groups of ten per cage and maintained in a regulated environment ($23 \pm 1°C$, $50 \pm 5\%$ humidity) with a 12:12 h light/dark cycle (lights on between 09 h 00 min and 21 h 00 min). Both laboratory chow and water were available *ad libitum* except during the period of water-deprivation or testing. The animals were allowed one week for adaptation to the laboratory conditions before the experiments. All experimental protocols were conducted with due regard for the Japanese Experimental Animal Research Association standards as defined in the Guidelines for Animal Experiments (1987), and were approved in advance by the Animal Research Committee at Nagoya University.

(+)-Eburnamenine-14-carboxylic acid (2-nitroxyethyl) ester (VA-045) was obtained from Taisho Pharmaceutical Co. Ltd. (Tokyo, Japan). The chemical structure is shown in Figure 1. VA-045 was dissolved in 2.5% (w/v) ascorbic acid solution in distilled water and four doses ($0.5-4.0$ mg kg^{-1}) were used in this experiment. All injections were made intraperitoneally in a volume of 0.1 ml 10 g^{-1} body weight. Dose and dosing regimens of VA-045 were based on our preliminary experiments. Solutions were freshly prepared each day.

Apparatus

The apparatus for the water-finding task was constructed as previously described (Nabeshima & Ichihara, 1993). Briefly, it consists of an open field ($30 \times 50 \times 15$ cm high) with an alcove ($10 \times 10 \times 10$ cm) in the middle of one wall of the enclosure. The inside was painted gray and the floor of the open field was divided into 15 identical squares with visible lines for measuring ambulatory activity. A metal drinking tube, identical to those used in their home cages, was inserted into the centre of the alcove ceiling.

The simple weight-drop device consists of a cylindrical-shaped acrylic weight (diameter 1 cm, length 20 cm, weight 21 g), a Plexiglas tube (inner diameter 14 mm), and a silicon rubber platform ($10 \times 10 \times 0.3$ cm high). The weight is held by a string which is attached to the upper part of the weight and allowed to made head impact through the tube by a dropping. Under the lower opening of the tube was the silicon rubber platform, on which the anaesthetized mouse was mounted by hand.

Production of mild TBI in mice

The procedures for production of experimental mild TBI in mice have been described previously (Tang *et al*., 1997a). Briefly, the mice were lightly anaesthetized with halothane (about 3% in air) in a small animal anaesthetizer (Model TK-4, Bio Machinery, Japan) and then placed in a prone position on the rubber platform. A blunt head shock was delivered by dropping the weight through the tube from a height of 25 cm, an injury level previously demonstrated to produce a concussive-like mild TBI without contusions, focal lesions and intraparenchymatous haemorrhage in the brain (Tang *et al*., 1997b). The duration of loss of righting reflex was recorded as the index for evaluating the condition of consciousness. Sham-injured mice were anaesthetized and placed in the prone position on the platform but did not receive the drop impact.

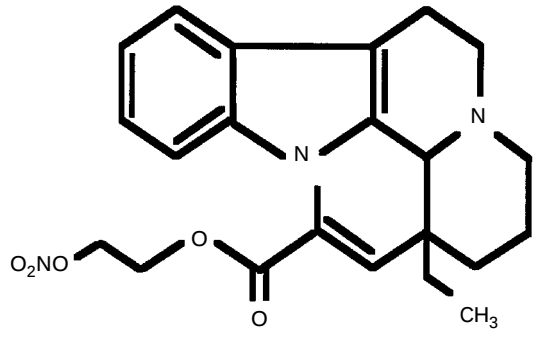

Figure 1 Chemical structure of (+)-eburnamenine-14-carboxylic acid (2-nitroxyethyl) ester (VA-045).

Procedures for the task examination

Since changes in either learning and memory or motor activities are sensitive to the measurements in water-finding task, we employed this task to evaluate effects of VA-045 in the present study. A novel procedure of water-finding task was used as described in our previous study (Tang *et al*., 1997c). Briefly, it consists of three sessions, acquisition trial, retention test and retest, with a 48 h interval between each session. In acquisition trial, the mice, which had not been deprived of water, were placed individually into one corner of the open field and allowed to freely explore the environment for 3 min. The elapsed time required to start exploration of the open field was recorded as starting latency and the elapsed time required to get into the alcove was recorded as entering latency. The frequency of touching, sniffing or licking the water tube in the alcove was recorded as number of approaches. Throughout the trial, ambulation was measured by counting the number of times the animal crossed from one square to another in the open field (*i.e*., locomotion count). Mice that did not approach the water tube during the 3 min test were excluded from the following experiment.

Following acquisition trial, animals were immediately returned to their home cages and maintained as usual with free access to water until to about 24 h before the retention test, which was carried out 48 h after the acquisition trial. In the retention test, the mice deprived of water for about 24 h in their home cages were again individually placed into the same corner of the apparatus and tested for 3 min. Besides starting latency, entering latency, and ambulation counts, drinking latency, defined as the elapsed time to start drinking water after the mouse had been placed into the apparatus, and finding latency, defined as the elapsed time to find the water spout after entering the alcove, were also recorded. The drinking latency of the mice that did not find the water spout within the 3 min test was recorded as 181 s.

A retest was used in this study to evaluate further the task performance. Following the retention test, mice were immediately returned to their home cages and maintained as usual with free access to water until to about 12 h before the retest. In our pilot studies, we found that a second 24 h water deprivation could produce to some extent deficits in the feeding-related motivation in both the normal as well as TBI mice. To minimize the interference of these deficits, we reduced the duration of the second water deprivation to about 12 h. In the retest, mice were individually tested in the open field as for the retention test and the behavioural parameters were recorded as for the retention test. In all experimental sessions, the floor of the open field was cleaned with 95% alcohol followed by water after each individual test.

Experimental procedures

All experiments were carried out between 10 h 00 min and 19 h 00 min in a sound-proofed room. In each test, mice were randomly allocated to treatment conditions in such a way that the investigators were blind to individual treatment. At first, we examined effects of VA-045 in naive mice to see whether this compound affects processes of learning and memory under normal conditions. For this purpose, VA-045 ($0.5-4.0$ mg kg^{-1}) was given immediately after the acquisition trial and the memory functions were evaluated in the retention test and retest.

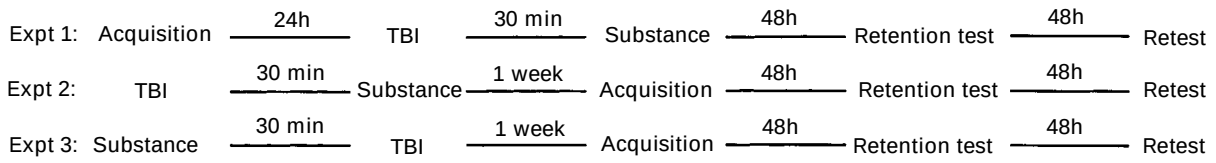

Figure 2 Experimental protocols.

After that, as shown in Figure 2, three experiments were designed to evaluate effects of VA-045 on the mild TBI-induced learning and memory deficits. Expt 1 was carried out to assess effects on retention deficit observed in TRA mice. Normal animals first received acquisition training and 24 h later, were subjected to TBI. Injured mice randomly received a single injection of VA-045 (0.5–4.0 mg kg^{-1}) or vehicle 30 min after TBI and the sham-injured mice were treated with vehicle. Memory functions were evaluated in retention test and retest. Expts 2 and 3 were conducted to determine whether VA-045 attenuates or prevents learning and retention deficits observed in TAA mice, respectively. In Expt 2, animals were first subjected to TBI and 30 min later, randomly treated with VA-045 (0.5–4.0 mg kg^{-1}) or vehicle. Sham-injury mice were treated with vehicle. In Expt 3, normal mice were randomly treated initially with either VA-045 (0.5–4.0 mg kg^{-1}) or vehicle and 30 min later, subjected to TBI or sham injury. All of the mice in the two experiments were allowed to recover for one week in their home cages and then subjected to the task examinations including acquisition trial, retention test and retest.

Gross histological examination (GHE)

Our previous studies have extensively characterized the histological changes in this model (Tang *et al*., 1997b). To confirm the homogeneity of the injury, five injured mice which had not received the chemical treatment and five sham-injury mice were subjected to the GHE 1 h after TBI. Following perfusion fixation, the scalp was carefully shaved for skull fracture examination. The skull was then carefully removed and every surface of the brain was examined for evidence of subdural or subarachnoid haemorrhages and focal brain lesion or contusions. A series of gross coronal and paramedian sagittal sections was made for a gross intraparenchymatous, intraventricular and periventricular haemorrhage examination.

Statistical analysis

Results are expressed as means ± s.e.mean. The differences in latencies, ambulation counts, number of approaches between TBI and the sham-injured mice were determined by one-way ANOVA test, followed by Dunnett's *post hoc* test. The significant level of the duration of loss of righting reflex between each TBI group was determined by Student's *t* test. The number of animals excluded from the acquisition trial was analysed by use of Fisher's exact probability test. Data were considered statistically significant at $P<0.05$ level.

Results

Effects of VA-045 on learning and memory in naive mice

No significant difference in the general behavioural parameters, including starting and entering latencies, number of approaches and ambulation counts, was found between each treatment condition in the acquisition trial (data not shown). As shown in Figure 3, latencies for finding and drinking but not for starting and entering of the VA-045-treated mice were slightly but significantly prolonged in the retention test ($F(4,67)=2.78$, $P<0.05$ and $F(4,67)=2.87$, $P<0.05$) and the retest ($F(4,67)=3.16$, $P<0.05$ and $F(4,67)=3.02$, $P<0.05$), compared to those of vehicle-treated mice. Further analyses indicated that significant effects were observed at doses of 2.0 mg kg^{-1} in the retention test ($P<0.05$ and $P<0.05$) and the retest ($P<0.05$ and $P<0.05$) and 4.0 mg kg^{-1} in the retention test ($P<0.05$ and $P<0.05$) and the retest ($P<0.05$ and $P<0.01$). Ambulatory activity was not significantly affected at the doses used in the retention test or the retest. These results indicate that the higher doses of VA-045 exerted a detrimental effect on memory functions in the naive mice.

Effects of VA-045 on retention deficit in TRA mice (Expt 1)

As shown in Table 1, no significant difference in the general behavioural parameters was found between each group before TBI in the acquisition trial. The number of animals excluded from the experiment without approach to the water tube during acquisition trial was less than 10% and no significant difference was found between each VA-045-treated group and the vehicle-treated group (data not shown). There was no significant difference in the values of the duration of loss of righting reflex after TBI between each TRA group (data not

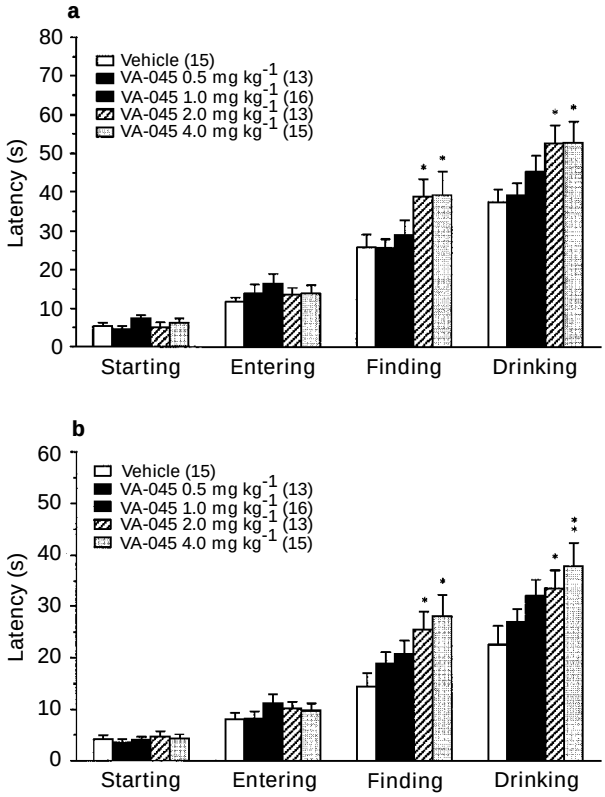

Figure 3 Effects of VA-045 administered immediately after acquisition trial in the normal mice in the retention test (a) and retest (b). Latencies for starting, entering, finding and drinking are shown as means ± s.e.mean; the number of animals in each group is shown in parentheses. $^{*}P<0.05$, $^{**}P<0.01$, compared to those of the vehicle-treated normal mice, determined by one-way ANOVA, followed by Dunnett's *post hoc* test.

shown). As shown in Figure 4, in comparison with those of the sham-injury mice, latencies for finding and drinking but not for starting or entering of the vehicle-treated TRA mice were significantly prolonged in the retention test ($F(1,35)=27.44$, $P<0.001$ and $F(1,35)=31.77$, $P<0.001$) and retest ($F(1,35)=27.43$, $P<0.001$ and $F(1,35)=31.12$, $P<0.001$), indicating that in TRA mice their task performance was dramatically impaired. VA-045 administered 30 min after TBI significantly attenuated the prolonged latencies for finding and drinking in the retention test ($F(4,81)=2.64$, $P<0.05$ and $F(4,81)=2.78$, $P<0.05$) and retest ($F(4,81)=3.54$, $P<0.05$ and ($F(4,81)=3.96$, $P<0.01$), compared to those of the vehicle-treated TRA mice. *Post-hoc* analyses indicated that significant effects were observed at doses of 0.5 mg kg^{-1} in the retention test ($P<0.01$ and $P<0.01$) and retest ($P<0.01$ and $P<0.01$) and 1.0 mg kg^{-1} in the retention test ($P<0.05$ and $P<0.05$) and retest ($P<0.01$ and $P<0.01$), indicating that VA-045 at the lower doses significantly attenuated the deficits of task performance observed in TAA mice.

Effects of VA-045 administered 30 min post-TBI on general behaviour in TAA mice (Expt 2)

There was no significant difference in the values of the duration of loss of righting reflex after TBI between each TAA group (data not shown). The general behaviour was evaluated in the acquisition trial. Following TBI, as shown in Table 2, no significant changes were found in the general behavioural parameters, except for entering latency ($F(1,34)=4.18$, $P<0.05$) and number of approaches ($F(1,34)=5.01$, $P<0.05$), compared to those of the sham-injured mice. VA-045 showed no obvious effects on these behaviours. Although a significant effect was observed on the increase in the number of approaches at the dose of 2.0 mg kg^{-1} ($P<0.05$) in the comparison between individual group, overall ANOVA analyses revealed no significant difference in each parameter between VA-045- and the vehicle-treated TAA mice. Ambulation counts were not significantly affected by either the TBI or the chemical treatment. The number of animals excluded from the acquisition trial without approach to the water tube was less than 10% and no significant difference was found between each VA-045-treated group and the vehicle-treated group (data not shown). These results indicate that the general behaviour, as a whole, was not dramatically affected by the mild TBI or VA-045.

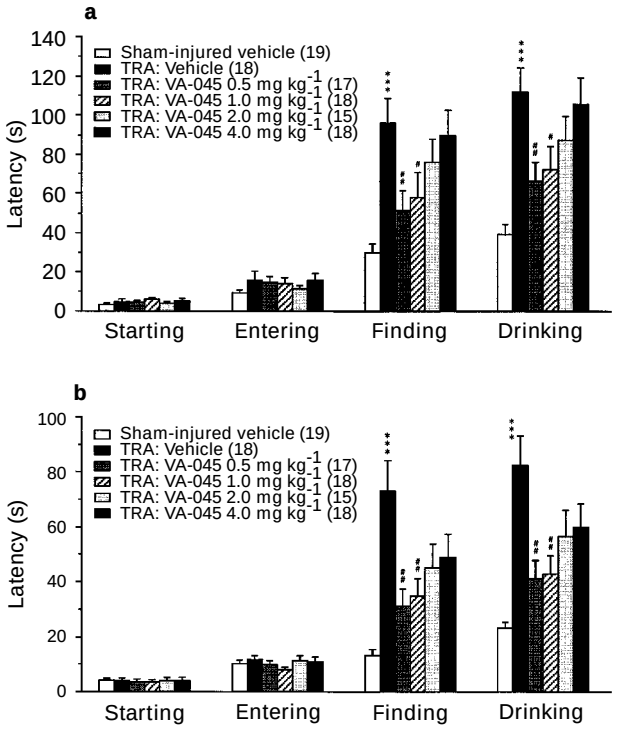

Figure 4 Effects of VA-045 administered 30 min post-trauma on retention deficit in TRA mice in the retention test (a) and retest (b). Latencies for starting, entering, finding, and drinking are shown as means ± s.e.mean, and the number of animals in each group is shown in parentheses. ***$P<0.001$, compared to those of the sham-injured mice; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared to those of the vehicle-treated TRA mice, determined by one-way ANOVA, followed by Dunnett's *post hoc* test.

Table 1 General behaviour during the acquisition trial in normal mice

Group	*Dose* (mg kg^{-1})	n	*Latency* (s) *Starting*	*Entering*	*No. of approaches to water tube*	*Ambulation* (counts)
Sham-injured						
Vehicle		19	8.11 ± 1.51	25.79 ± 5.13	7.26 ± 0.74	31.63 ± 5.43
TBI						
Vehicle		18	8.39 ± 1.32	24.44 ± 5.97	6.78 ± 0.62	32.39 ± 4.96
VA-045	0.5	17	7.18 ± 1.43	18.59 ± 1.68	8.47 ± 0.67	32.18 ± 7.11
VA-045	1.0	18	10.06 ± 1.87	25.00 ± 3.62	6.06 ± 0.38	28.88 ± 4.41
VA-045	2.0	15	8.18 ± 1.91	26.12 ± 5.52	8.71 ± 1.27	25.18 ± 5.47
VA-045	4.0	18	7.88 ± 1.09	25.53 ± 2.83	7.47 ± 0.90	31.88 ± 4.12

Values show the means ± s.e.mean.

Table 2 Effects of VA-045 administered 30 min post-trauma on general behaviour during the acquisition trial in TAA mice

Treatment	*Dose* (mg kg^{-1})	n	*Latency* (s) *Starting*	*Entering*	*No. of approaches to water tube*	*Ambulation* (counts)
Sham-injured						
Vehicle		19	9.53 ± 1.56	27.95 ± 4.78	6.79 ± 0.80	30.21 ± 4.27
TAA						
Vehicle		17	13.94 ± 2.35	44.59 ± 6.75*	4.53 ± 0.59*	25.76 ± 4.48
VA-045	0.5	18	11.17 ± 1.47	32.61 ± 5.18	6.28 ± 0.76	25.67 ± 3.64
VA-045	1.0	19	9.89 ± 1.52	41.21 ± 6.72	6.32 ± 0.93	27.63 ± 4.44
VA-045	2.0	20	12.50 ± 2.44	38.75 ± 5.08	6.90 ± 0.71#	23.40 ± 3.06
VA-045	4.0	19	13.63 ± 2.98	44.32 ± 7.86	5.68 ± 0.86	26.47 ± 3.48

Values show the means ± s.e.mean. TAA: TBI-induced anterograde amnesia. *$P<0.05$, compared to those of the sham-injured mice. #$P<0.05$, compared to those of the vehicle-treated TAA mice.

Effects of VA-045 on deficits of learning and retention in TAA mice (Expt 2)

Compared to those of the sham-injured mice, as shown in Figure 5, latencies for finding and drinking but not for starting or entering of the vehicle-treated TAA mice were dramatically prolonged in the retention test ($F(1,34)=39.5$, $P<0.001$ and $F(1,34)=44.64$, $P<0.001$) and the retest ($F(1,34)=22.98$, $P<0.001$ and $F(1,34)=23.99$, $P<0.001$), indicated that TAA mice were dramatically impaired in the task performance. VA-045 administered 30 min after TBI significantly shortened the prolonged latencies for finding and drinking in the retention test ($F(4,88)=3.424$, $P<0.05$ and $F(4,88)=3.31$, $P<0.05$) and retest ($F(4,88)=4.08$, $P<0.01$ and $F(4,88)=3.93$, $P<0.01$), compared to those of the vehicle-treated TAA mice. *Post-hoc* analyses indicated that significant effects were observed, at doses of 1.0 mg kg^{-1} on the retention test ($P<0.05$ and $P<0.05$) and the retest ($P<0.05$ and $P<0.01$), and 2.0 mg kg^{-1} on the retention test ($P<0.01$ and $P<0.01$) and retest ($P<0.01$ and $P<0.01$). These data indicate that VA-045 significantly attenuates the deficits of task performance observed in TAA mice.

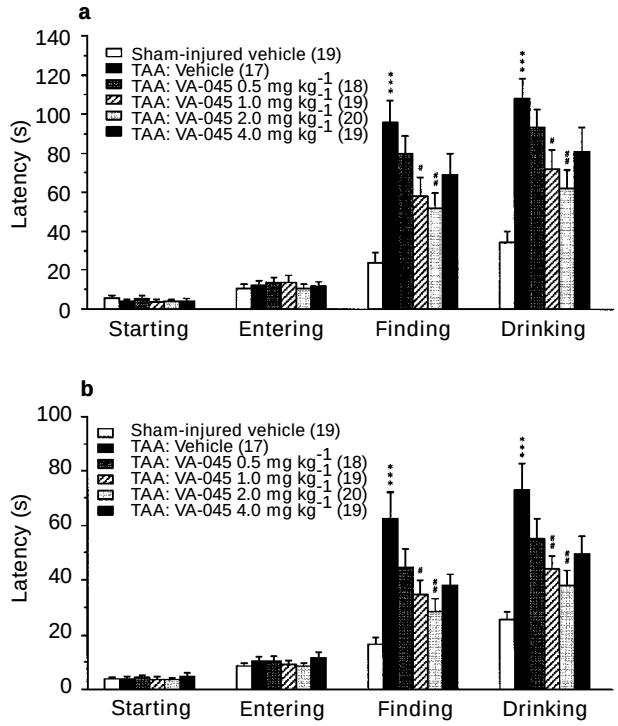

Figure 5 Effects of VA-045 administered 30 min post-trauma on the deficits of learning and retention in TAA mice in the retention test (a) and retest (b). Latencies for starting, entering, finding, and drinking are shown as means ± s.e.mean, and the number of animals in each group is shown in parentheses. ***$P<0.001$, compared to those of the sham-injured mice; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared to those of the vehicle-treated TAA mice, determined by one-way ANOVA, followed by Dunnett's *post hoc* test.

Effects of VA-045 administered 30 min before TBI on general behaviour in TAA mice (Expt 3)

There was no significant difference in the values for the duration of loss of righting reflex after TBI between each TAA group (data not shown). As shown in Table 3, a significant decrease in number of approaches ($F(1,30)=7.49$, $P<0.05$) was observed in TAA mice in the acquisition trial, compared to that of the sham-injured mice. VA-045 showed no obvious effects on these behaviours, since overall ANOVA analyses revealed no significant difference in each parameter between the VA-045- and the vehicle-treated TAA mice, except that a significant effect on the increased number of approaches was found at the dose of 2.0 mg kg^{-1} ($P<0.05$) in the individual group comparison. Ambulation counts were significantly affected by neither the TBI nor the chemical treatment. The number of animals excluded from the acquisition trial without approach to the water tube was less than 10% and no significant difference was found between each VA-045-treated and the vehicle-treated group (data not shown). These results indicate that the general behaviour, as a whole, was not dramatically affected by the mild TBI and VA-045 had little effect on this behaviour.

Effects of VA-045 on prevention from learning and memory deficits associated with TBI in mice (Expt 3)

As shown in Figure 6, similar to the results of Expt 2 (Figure 5), latencies for finding and drinking but not for starting or entering of the vehicle-treated TBI mice were significantly prolonged in the retention test ($F(1,30)=26.37$, $P<0.001$ and $F(1,30)=27.44$, $P<0.001$) and retest ($F(1,30)=33.27$, $P<0.001$ and $F(1,30)=34.03$, $P<0.001$), indicating that mild TBI dramatically impaired the task performance. VA-045 administered 30 min before TBI significantly attenuated the delay of latencies for finding and drinking in the retention test ($F(4,78)=2.90$, $P<0.05$ and $F(4,78)=2.51$, $P<0.05$) and retest ($F(4,78)=3.89$, $P<0.01$ and $F(4,78)=3.75$, $P<0.01$), compared to those of the vehicle-treated TBI animals. Further analyses of individual group data indicated that significant effects were found at doses of 0.5 mg kg^{-1} in the retention test ($P<0.01$ and $P<0.01$) and retest ($P<0.01$ and $P<0.01$), 1.0 mg kg^{-1} in the retention test ($P<0.01$ and $P<0.01$) and retest ($P<0.01$ and $P<0.01$) and 2.0 mg kg^{-1} in the retention test ($P<0.05$ and $P<0.05$) and retest ($P<0.05$ and $P<0.05$), indicating that VA-045 significantly protects against TBI-induced deficits in the task performance in mice.

Summary of the effects of TBI and VA-045 on ambulatory activities in the retention test and retest in TRA and TAA mice (Expt 1–3)

To test further the hypothesis that motor changes following TBI or chemical treatment could contribute to the observed

Table 3 Effects of VA-045 administered 30 min pre-trauma on general behaviour during the acquisition trial in TAA mice

Treatment	*Dose* (mg kg^{-1})	n	*Latency* (s) *Starting*	*Entering*	*No. of approaches to water tube*	*Ambulation* (counts)
Sham-injured						
Vehicle		15	6.89 ± 0.80	22.13 ± 2.73	7.93 ± 0.73	33.87 ± 4.64
TAA						
Vehicle		17	8.76 ± 1.99	27.12 ± 4.30	5.29 ± 0.63*	29.12 ± 5.67
VA-045	0.5	17	9.88 ± 1.92	31.41 ± 5.45	5.35 ± 0.80	27.29 ± 4.41
VA-045	1.0	16	8.50 ± 1.31	29.13 ± 4.80	5.38 ± 0.64	30.94 ± 4.55
VA-045	2.0	17	7.76 ± 1.35	29.94 ± 5.36	7.65 ± 0.74$^{\#}$	30.12 ± 2.81
VA-045	4.0	16	9.00 ± 1.93	34.64 ± 7.84	5.75 ± 0.65	35.38 ± 5.69

Values show the means ± s.e.mean. TAA: TBI-induced anterograde amnesia. *$P<0.05$, compared to those of the sham-injured mice. $^{\#}P<0.05$, compared to those of the vehicle-treated TAA mice.

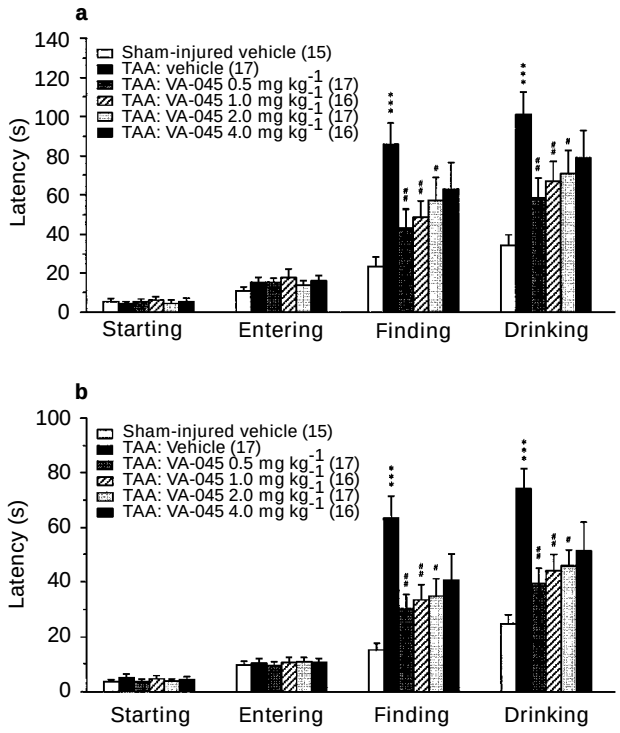

Figure 6 Effects of VA-045 administered 30 min pre-trauma on prevention of learning and memory deficits after TBI in mice in the retention test (a) and retest (b). Latencies for starting, entering, finding, and drinking are shown as means ± s.e.mean, and the number of animals in each group is shown in parentheses. $^{***}P<0.001$, compared to those of the sham-injured mice; $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared to those of the vehicle-treated TBI mice, determined by one-way ANOVA, followed by Dunnett's *post hoc* test.

behavioural effects of the treatment, we measured ambulatory activities in the same apparatus as the one used for the behavioural task. The effects of the TBI and VA-045 on the ambulatory activities are summarized in Table 4. A thorough statistic analysis revealed no significant difference between TBI mice and the sham-injured mice, although the insult of TBI had a tendency to lower ambulation counts in both the retention test and retest. VA-045 administered 30 min post-injury in TRA or TAA mice showed a tendency to increase ambulation counts in the retention test, while an overall ANOVA analysis revealed no significant difference between the VA-045- and the vehicle-treated TBI mice. In addition, this tendency disappeared in the retest in either TRA or TAA mice. These results indicate that the TBI and VA-045 have no significant effects on ambulatory activity.

GHE findings

Gross histopathological changes were examined 1 h after TBI. The brains looked normal without contusion or focal lesion. No skull fracture was found in any injured mouse. No focal contusion and subdural or subarachnoid haemorrhage was found. Examination of a series of gross coronal and paramedian sagittal sections revealed that there was no haemorrhage in the ventricles. Intraparenchymatous haemorrhage was not found in the areas examined, including the cortex, hippocampus, corpus callosum, thalamus, cerebellum and brain-stem.

Discussion

In the present study, we examined the effects of (+)-eburnamenine-14-carboxylic acid (2-nitroxyethyl) ester (VA-045), a novel apovincaminic acid derivative, on learning and memory deficits associated with mild TBI in mice. The results showed that VA-045 significantly attenuates or protects against the TBI-induced deficits in learning and memory without obvious effects on motor activities. It is concluded that VA-045 may be useful for the acute pharmacological treatment of post-traumatic learning and memory deficits. This is the first study to demonstrate effects of a compound on retrograde and anterograde memory dysfunction in a mild TBI mouse model.

By definition, retrograde amnesia is characterized by a deficit in memory retention, whereas anterograde amnesia by both deficit in learning and a delayed retention process (Rutherford *et al*., 1977; Rimel *et al*., 1982; Squire & Cohen, 1984). In this study, retrograde and anterograde amnesic mice were experimentally reproduced by means of post- and pre-acquisition head injury, respectively. In TRA mice, since the acquisition trial was given before trauma, learning was intact during the acquisition trial. The poor score of the task performance in retention test therefore resulted from the impaired retention process but not from the learning itself. However, in TAA mice, since acquisition trial was given after trauma, learning might be inhibited by the insult of TBI. The poor scores of the task performance in the retention test should be reasoned from either the impaired learning or the delayed retention process. These features are consistent with the definitions of retrograde and anterograde amnesia in the clinical situation.

Memory functions were further evaluated in a retest in this study. Since the animals which were previously water-deprived

Table 4 Effects of VA-045 administered 30 min post- or pre-trauma on ambulatory activity in the retention test and retest in TRA and TAA mice

VA-045 administered 30 min post-trauma in TRA mice

Treatment	*Dose* (mg kg^{-1})	n	*Ambulation* (counts) *Retention test*	*Retest*
Sham-injured				
Vehicle		19	19.34 ± 3.05	16.24 ± 3.45
TRA				
Vehicle		18	18.21 ± 1.38	14.55 ± 3.19
VA-045	0.5	17	16.38 ± 2.45	18.92 ± 3.89
VA-045	1.0	18	22.89 ± 2.18	11.23 ± 2.09
VA-045	2.0	15	19.32 ± 2.85	10.21 ± 1.73
VA-045	4.0	18	15.38 ± 2.75	17.45 ± 3.24

VA-045 administered 30 min post-trauma in TAA mice

Treatment	*Dose* (mg kg^{-1})	n	*Ambulation* (counts) *Retention test*	*Retest*
Sham-injured				
Vehicle		19	23.93 ± 2.30	18.80 ± 2.70
TAA				
Vehicle		17	15.31 ± 1.06	11.29 ± 1.52
VA-045	0.5	18	18.88 ± 3.81	9.65 ± 1.37
VA-045	1.0	19	25.25 ± 2.38	10.06 ± 1.69
VA-045	2.0	20	17.41 ± 2.50	14.12 ± 2.38
VA-045	4.0	19	19.69 ± 3.93	13.06 ± 2.07

VA-045 administered 30 min pre-trauma in TAA mice

Treatment	*Dose* (mg kg^{-1})	n	*Ambulation* (counts) *Retention test*	*Retest*
Sham-injured				
Vehicle		15	20.43 ± 4.29	15.03 ± 2.66
TAA				
Vehicle		17	18.34 ± 3.53	10.47 ± 1.44
VA-045	0.5	17	14.73 ± 2.18	9.67 ± 1.18
VA-045	1.0	16	15.28 ± 1.98	10.95 ± 1.07
VA-045	2.0	17	13.45 ± 2.08	9.20 ± 1.15
VA-045	4.0	16	19.74 ± 3.67	8.53 ± 1.15

Values show the means ± s.e.mean. TRA: TBI-induced retrograde amnesia; TAA: TBI-induced anterograde amnesia.

received a water reward in the retention test, this will also engage memory processes and the rewarding effects can be further analysed by a re-examination, termed retest in this study. We have reasons to consider that the outcome of the retest is related to the retention or retrieval system. A poor score in the retest might indicate a prolonged retention deficit or impairment in retrieval system in both TRA and TAA mice. By analysing the data from either retention test or retest, we concluded that VA-045 is effective for the improvement or protection from learning and memory deficits observed in this TBI model. These results provide additional evidence to support the view that certain suitable pre- or post-trauma chemical treatments are beneficial for the recovery from the cognitive deficits associated with TBI (Hayes *et al*., 1989; McLean *et al*., 1991; Pierce *et al*., 1993).

It is not clear why administration of VA-045 before or soon after trauma can get such effects. VA-045 is a derivative of apovincaminic acid (Kawashima *et al*., 1993), a major metabolite of vinpocetine in the body (Miskolczi *et al*., 1990). Since it is able to produce vasodilatation, vinpocetine was once used broadly to treat cerebravascular disease (Otomo *et al*., 1985). In the laboratory, vinpocetine has been found to reduce ischaemia-induced neurone loss in the hippocampus (Rischke & Krieglstein, 1991). With similar structure to vinpocetine, VA-045 not only shares some biological activities with it but also possesses greater potency, such as increase of cerebral blood flow (Kawashima *et al*., 1993; Okuyama *et al*., 1994). Post-traumatic ischaemia is one of the major cause of the neurological sequelae (Cortez *et al*., 1989; Yamakami & McIntosh, 1989; Dietrich *et al*., 1994), the effects of VA-045 observed in this study might be due to its effects on cerebral blood flow.

However, it should be noted that VA-045 at the higher doses in this study exerted a detrimental effect on learning and memory in the naive animals (Figure 3), indicating that this compound may interfere with the processes of learning and memory. Therefore, some other mechanisms may exist in the regulation of the effects of VA-045. Analyses of the dose-effect responses also support this suggestion. In this study, the effects of VA-045 were observed only in a narrow dose range in either TRA or TAA mice, in contrast to the observations in another experimental paradigm, in which the effects of VA-045 on aged-related memory deficits were found for a wide dose range (Okuyama *et al*., 1994). This finding might imply that the effects were not mainly due to its action on cerebral blood flow. In TRA mice, significant effects were observed with the lower doses used (Figure 4). It is unlikely that the lower doses exert effects on cerebral blood flow while the higher doses do not. In addition, the finding that the effects were inversely related to the doses in a different model do not support the possibility that an increased cerebral blood flow is responsible for the effects of VA-045 on learning and memory. Thus, the mechanism of action of VA-045 seems rather complex.

It is well known that neurotransmitter systems are involved in the processes of learning and memory. Following TBI, changes in these systems can be divided into acute and chronic responses. During the acute response, over-release of neurotransmitters such as acetylcholine (ACh), dopamine, as well as glutamate, contribute to the production of memory deficits (Faden *et al*., 1989; Gorman *et al*., 1989; Nilsson *et al*., 1990; McIntosh *et al*., 1994). This hypothesis has been confirmed by a great number of experiments, in which effects were obtained by administration of antagonists for ACh (Ward, 1950; Lyeth *et al*., 1988), glutamate (for review see Okiyama *et al*., 1995; Smith *et al*., 1993), or dopamine (Tang *et al*., 1997c) soon after trauma in terms of acute pharmacological treatment. In this study, VA-045 was administered 30 min pre- or post-trauma and the half-life of the compound is only about 3–4 h (unpublished data from Taisho Pharmaceutical Co. Ltd), the results therefore showed an acute pharmacological action. Furthermore, not only has vinpocetine been shown to have specific glutamate receptor antagonistic properties, such as inhibition of the release of ACh and dopamine evoked by stimulation of glutamate receptors (Kaneko *et al*., 1991; Kiss *et al*., 1991) and to protect against the cytotoxicity of glutamate in neuronal cells (Miyamoto *et al*., 1989), but VA-045 has also been found to attenuate memory deficits associated with disturbance in cholinergic activities (Ogawa *et al*., 1992). So it is likely that the effects of VA-045 are dependent, at least partly, on an interaction with neurotransmitter system(s). Analyses of the dose-response curves were also consistent with this suggestion. Since an acute disturbance of the neurotransmitter systems is a crucial event in the development of learning and memory deficits (Hayes *et al*., 1989; Dunn-Meyall *et al*., 1994; McIntosh *et al*., 1994), it seems reasonable that only a 'most effective' interaction between an exogenous agent and specific neurotransmitter(s) in the brain could be 'most beneficial' for this disturbance and subsequently lead to an improved behavioural response. The finding that the effects of VA-045 were only observed in a narrow dose range and a typical "U' shaped dose-effect curve was obtained in the TAA mice in this and other studies support this hypothesis. The finding that the effects were inversely related to the doses implies a complex interaction between VA-045 and neurotransmitter systems following TBI. This interaction is not only dependent on the dose of exogenous agent, but also on the events in the processes of learning and memory.

In conclusion, the present study demonstrated that post- or pre-trauma administration of VA-045 significantly ameliorated or prevented learning and memory deficits associated with a mild TBI in the mice. VA-045 may be a strong candidate for the acute pharmacological treatment of post-traumatic learning and memory deficits, although the mechanisms remain to be studied further.

This research was supported by grant-in-aids for Gerontological Science Research from the Ministry of Health and Welfare, Japan (94A-2405), from the Ministry of Education, Science and Culture, Japan (07557009), and from the Uehara Memorial Foundation Grant, and an SRF Grant for Biomedical Research. We thank Taisho Pharmaceutical Co. Ltd., Tokyo, Japan, for providing VA-045.

References

BINDER, L. (1986). Persistent symptoms after mild head injury: a review of the postconcussive syndrome. *J. Clin. Exp. Neuropsychol*., **8,** 323–346.

CORTEZ, S.C., MCINTOSH, T.K. & NOBLE, L.J. (1989). Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations. *Brain Res*., **482,** 271–282.

DIETRICH, W.D., ALONSO, O. & HALLEY, M. (1994). Early microvascular and neuronal consequences of traumatic brain injury: A light and electron microscopic study in rats. *J. Neurotrauma*, **11,** 289–301.

DUNN-MEYALL, A., PAN, S. & LEVIN, B.E. (1994). Focal traumatic brain injury cause widespread reductions in rat brain norepinephrine turnover from 6 to 24 hr. *Brain Res*., **660,** 88–95.

FADEN, A.I., DEMEDIUK, P., PANTER, S.S. & VINK, R. (1989). The role of excitatory amino acid receptors and NMDA receptors in traumatic brain injury. *Science*, **244,** 798–800.

GABRIEL, H.I. (1985). Approaches to the analysis of the neural bases of memory, In *Memory, Imprinting, and the Brain: Inquiry into Mechanisms*. Oxford Psychology Series No. 1, pp. 1–28. Oxford: Clarendon Press.

GORMAN, L.K., SHOOK, B.L. & BECKER, D.P. (1993). Traumatic brain injury produces impairments in long-term and recent memory. *Brain Res*., **614,** 29–36.

GORMAN, L.K., FU, K., HOVDA, D.A., BECKER, D.P. & KATAYAMA, Y. (1989). Analysis of acetylcholine release following concussive brain injury in the rat. *J. Neurotrauma*, **6,** 203–209.

HAYES, R.L., LYETH, B.G. & JENKINS, L.W. (1989). Neurochemical mechanisms of mild and moderate head injury: implications for treatment. In *Mild Head Injury*. ed. Levin, H.S., Eisenberg, H.M. & Benton, A.L. pp. 54–79. Oxford: Oxford University Press.

KANEKO, S., SUGIMURA, M., INOUE, T. & SATOH, M. (1991). Effects of several drugs on NMDA channel function: evaluation using Xenopus oocytes and [3H] MK-801 binding. *Eur. J. Pharmacol*., **207,** 119–128.

KAWASHIMA, Y., IKEMOTO, T., HORIIGUCHI, A., HAYASHI, M., MATSMOTO, K., KAWARASAKI, K., YAMAZAKI, R., OKUYAMA, S. & HATAYAMA, K. (1993). Synthesis and pharmacological evaluation of (nitrooxy)alkyl apovincaminates. *J. Med. Chem*., **36,** 815–819.

KISS, B., CAI, N.S. & ERDE, S.L. (1991). Vinpocetine preferentially antagonizes quisqualate/AMPA receptor responses: evidence from release and ligand binding studies. *Eur. J. Pharmacol*., **209,** 109–112.

LEVIN, H.S. (1990) Memory deficit after closed head injury. *J. Clin. Exp. Neuropsychol*., **12,** 129–153.

LEVIN, H.S., MATTIS, S., RUFF, R., EISENBERG, H., MARSHALL, C., TOBAADOR, K., HIGH, W. & FRANKOWSKI, R. (1987). Neurobehavioral outcome following head injury: three-center study. *J. Neurosurg*., **65,** 234–243.

LYETH, B.G., DIXON, C.E., JENKINS, L., WHAMM, R.J., ALBERICO, A., YOUNG, H.F., STONNINGTON, H.H. & HAYES, R.L. (1988). Effects of scopolamine treatment on long-term behavioral deficits following concussive brain injury to the rat. *Brain Res*., **452,** 39–48.

MCINTOSH, T.K., YU, T. & GENNARELLI, T.A. (1994). Alteration in regional brain catecholamine concentrations after experimental brain injury in the rat. *J. Neurochem*., **63,** 1426–1433.

MCLEAN, Jr. A., CARDENAS, D.D., BURGESS, D. & GAMZU, E. (1991). Placebo-controlled study of pramiracetam in young males with memory and cognitive problems resulting from head injury and anoxia. *Brain Injury*, **5,** 375–380.

MISKOLCZI, P., KOZMA, K., POLGAR, M. & VERECZKEY, L. (1990). Pharmacokinetics of vinpocetine and its main metabolite apovincaminic acid before and after the chronic oral administration of vinpocetine to humans. *Eur. J. Drug Metab. Pharmacokinet*., **15,** 1–5.

MIYAMOTO, M., MURPHY, T.H., SCHNAAR, R.L. & COYLE, J.T. (1989). Antioxidants protect against glutamiate-induced cytotoxicity in a neuronal cell line. *J. Pharmacol. Exp. Ther*., **250,** 1132–1137.

NABESHIMA, T. & ICHIHARA, K. (1993). Measurements of dissociation of amnesic and behavioral effects of drugs in mice, In *Methods in Neurosciences*, Vol. 14, ed. Conn, P.M. pp. 217–229. New York: Academic Press.

NILSSON, P., HILLERED, L., PONTEN, U. & UNGERSTEDT, U. (1990). Changes in cortical extracellular levels of energy-related metabolites and amino acids following brain injury in rats. *J. Cerebral Blood Flow Metab*., **10,** 631–641.

OGAWA, S., OKUYAMA, S. & OTOMO, S. (1992). VA-045, a novel apovincaminic acid derivative, improved cholinergic deficits-induced amnesia in rats. *Clin. Neuropharmacol*., **15**(suppl. 1), 563B.

OKIYAMA, K., SMITH, D.H., GENNARELLI, T.A., SIMON, R.P., LEACH, M. & MCINTOSH, T.K. (1995). The sodium channel blocker and glutamate release inhibitor BW1003C87 and magnesium attenuate regional cerebral edema following experimental brain injury in the rat. *J. Neurochem*., **64,** 802–9.

OKUYAMA, S., HASHIMOTO-KITSUKAWA, S., OGAWA, S-I., IMAGAWA, Y., KAWASHIMA, K., KAWASHIMA, Y., ARAKI, H. & OTOMO, S. (1994). Effects of VA-045, a novel apovincaminic acid derivative, on age-related impairment evidence in electroencephalograph, caudate spindle, a passive avoidance task and cerebral blood flow in rats. *Gen. Pharmacol*., **25,** 1311–1320.

OTOMO, E., ATARASHI, J., ARAKI, G., ITO, E., OMAE, T., KUZUYA, F., NAKADA, T. & EBI, O. (1985). Comparison of vinpocetine with ifenprodil and dihydroergotoxin mesylate treatment and results of long treatment of vinpocetine. *Curr. Ther. Res*., **37,** 811–821.

PIERCE, J.E.S., SMITH, D.H., EISON, M.S. & MCINTOSH, T.K. (1993). The nootropic compound BMY-21502 improves spatial learning ability in brain injured rats. *Brain Res*., **624,** 199–208.

RIMEL, R.W., GIORDABI, B., BARTH, J. & JANE, J.A. (1982). Moderate head injury: completing the clinical spectrum of brain injury. *Neurosurgery*, **11,** 344–351.

RISCHKE, R. & KRIEGLSTEIN, J. (1991). Protective effect of vinpocetine against brain damage caused by ischemia. *Jpn. J. Pharmacol*., **56,** 349–356.

RUSSELL, W.R. (1935). Amnesia following head injuries. *Lancet*, **2,** 762–763.

RUTHERFORD, W.H., MERRETT, J.D. & MCDORALD, J.R. (1977). Sequelae of concussive caused by minor head injury. *Lancet*, **1,** 1–4.

SMITH, D.H., OKIYAMA, K., THOMAS, M.J. & MCINTOSH, T.K. (1993). Effects of the excitatory amino acid receptor antagonists kynurenate and indole-2-carboxylic acid on behavioral and neurochemical outcome following experimental brain injury. *J. Neurosci*., **13,** 5383–5392.

SQUIRE, L.R. (1986). Mechanisms of memory. *Science*, **232,** 1612–1619.

SQUIRE, L.R. & COHEN, N.J. (1984). Human memory and amnesia, In *Neurobiology of Learning and Memory*, ed. Lynch, G. pp. 3–64. Guilford: Guilford Press.

TANG, Y.-P., NODA, Y., HASEGAWA, T. & NABESHIMA, T. (1997a). A concussive-like brain injury model in mice (I): impairments in learning and memory. *J. Neurotrauma*, (in press).

TANG, Y.-P., NODA, Y., HASEGAWA, T. & NABESHIMA, T. (1997b). A concussive-like brain injury model in mice (II): selective neuron loss in the cortex and hippocampus. *J. Neurotrauma*, (in press).

TANG, Y.-P., NODA, Y. & NABESHIMA, T. (1997c). Involvement of activation of dopaminergic neuronal system in learning and memory deficits associated with experimental mild traumatic brain injury (TBI). *Eur. J. Neurosci*., (in press).

WARD, A. JR. (1950). Atropine in the treatment of closed head injury. *J. Neurosurg*., **7,** 398–402.

YAMAKAMI, I. & MCINTOSH, T.K. (1989). Effects of traumatic brain injury on regional cerebral blood flow in rats as measured with radiolabeled microspheres. *J. Cereb. Blood Flow Metab*., **9,** 117–241.

(Received December 10, 1996
Revised June 10, 1997
Accepted June 19, 1997)