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Effect of alaptide, its analogues and oxiracetam on memory for an elevated plus-maze in mice

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Abstract

In the present study, the elevated plus-maze was used to evaluate memory in female mice. In Experiment 1, the mice retested on day 1, 4 or 7 after the initial session escaped from the open arm into the enclosed arm in a significantly shorter time than those retested on day 10 or 14. Thus, a 10-day inter-session interval was chosen for testing drugs which were expected to enhance memory. In Experiment 2, in the retest performed on day 10, both alaptide (cyclo(L-alanyl-1-amino-1-cyclopentanecarbonyl)) and oxiracetam, given immediately after the 1st session, reduced the transfer latency from the open arm into the enclosed arm as compared with that of the controls. In Experiment 3, a similar effect, i.e., the retention of spatial information, was facilitated by post-session injections of 5 out of 21 alaptide analogues. The new compounds represent the 2,5-piperazinedione derivatives which contain 1-amino-1-cyclo-alkanecarboxylic acid (C3 to C7 ring). The cyclopentane- and cyclohexane-ring was substituted by an alkyl group. In the series with the cycloalkane ring, the importance of the structure of alaptide was confirmed again, which underlines the importance of the cyclopentane ring; the active structures had L-alanine instead of glycine as the second amino acid. Isomers of the cyclohexane series which contained methyl or *tert*-butyl were most active when the substitution was at position 3. Our results demonstrate that the model of long-term memory can be used to discriminate between closely related chemical structures.

Keywords: Alaptide; Cyclodipeptide; 2,5-Piperazinedione; Elevated plus-maze test; Memory; Oxiracetam; (Mouse)

1. Introduction

The history of the possible involvement of neuropeptides in learning and memory processes was opened by the findings of De Wied and colleagues (see, for example, De Wied, 1965, 1984, 1989; De Wied et al., 1975, 1984), who demonstrated that vasopressin and adrenocorticotropic hormone (ACTH) improve consolidation and retrieval in rodents. Vasopressinergic modulation of social recognition in rats has also been proved (Dantzer et al., 1987; Dantzer and Bluthé, 1993). Nicolaides et al. (1986) have suggested the possibility that vasopressin may serve as a precursor of smaller active peptides. Another peptide that has excited interest as a potential learning and memory enhancing agent is the tripeptide L-prolyl-L-leucyl-glycine amide, isolated as a hypothalamic factor inhibiting the release of melanocyte-stimulating hormone (MIF). In animals, MIF prevented puromycin-induced amnesia (Walter et al., 1975), delayed the extinction of active avoidance (Walter et al., 1978), and positively affected learning abilities in passive avoidance tests (Krejčí et al., 1980). By modification of the MIF molecule a spirocyclic dipeptide cyclo-(L-alanyl-1-amino-1-cyclopentanecarbonyl), termed alaptide (VÚFB 15754), has been prepared (Rádl et al., 1990). The beneficial effect of alaptide on learning and memory performance in rats and mice has been estimated in various experimental paradigms (Krejčí et al., 1986a,b; Hliňák et al., 1990; Hliňák and Krejčí, 1991a,b,1992). We have postulated that alaptide exerts a long-lasting effect and can influence all phases of the memory process. Alaptide is now under development as a nootropic drug for possible oral administration.

Therefore, we investigated the effect of various alaptide derivatives I (see Table 1) in which the size of the ring was modified from cyclopropane to cycloheptane with combination of second chiral amino acid. Furthermore, analogues which contained an alkyl group on the ring were also investigated. The present study was carried out with

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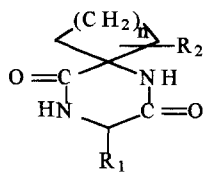
Table 1
Derivatives of 2,5-piperazinedione



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Compound	n	R_1	R_2
c(Gly-Apr)	1	H	H
c(Ala-Apr)	1	CH_3	H
c(Gly-Abu)	2	H	H
c(Ala-Abu)	2	CH_3	H
c(Gly-Ach)	4	H	H
c(Gly-Acp)	3	H	H
c(3-MeAch-D-Ala)	4	CH_3	3-CH_3
c(3-MeAch-Gly)	4	H	3-CH_3
c(3-MeAch-L-Ser)	4	CH_2OH	3-CH_3
c(3-MeAch-D-Ser)	4	CH_2OH	3-CH_3
c(3-MeAch-L-Val)	4	$CH(CH_3)_2$	3-CH_3
c(3-MeAch-D-Val)	4	$CH(CH_3)_2$	3-CH_3
c(3-MeAch-D-Phe)	4	$CH_2C_6H_5$	3-CH_3
c(3-MeAch-L-Phe)	4	$CH_2C_6H_5$	3-CH_3
c(L-Ala-2-MeAcp)	3	CH_3	2-CH_3
c(Ach-L-Ala)	4	CH_3	H
c(Ala-Achpt)	5	CH_3	H
c(3-MeAch-D-Leu)	4	$CH_2CH(CH_3)_2$	H
c(4-MeAch-Gly	4	H	4-CH_3
c(4-*t*BuAch-L-Ala)	4	CH_3	4-$C(CH_3)_3$
c(4-*t*BuAch-Gly)	4	H	4-$C(CH_3)_3$

Apr, 1-amino-1-cyclopropanecarbonyl; Abu, 1-amino-1-cyclobutanecarbonyl; Acp, 1-amino-1-cyclopentanecarbonyl; Ach, 1-amino-1-cyclohexanecarbonyl; Achpt, 1-amino-1-cycloheptanecarbonyl; 3-MeAch, 1-amino-3-methyl-1-cyclohexanecarbonyl; 2-MeAcp, 1-amino-2-methyl-1-cyclopentanecarbonyl; 4-MeAch, 1-amino-4-methyl-1-cyclohexanecarbonyl; 4-*t*BuAch, 1-amino-4-*tert*-butyl-1-cyclohexanecarbonyl.

the aim of determining the effect of alaptide analogues on learning and memory in mice. For this purpose, we chose the elevated plus-maze test as an experimental paradigm. The test was originally used for measurement of anxiety (Pellow et al., 1985; Lister, 1987). Recently, the paradigm was successfully used for evaluation of working memory in mice (Itoh et al., 1990, 1991). These authors have shown that the transfer latency (i.e., the time it took for the mice to move from the open arm to the enclosed arm) on the 2nd session was significantly prolonged in animals administered scopolamine immediately after the 1st session performed 24 h before, and that the prolongation of transfer latency was reversed by pre-treatment with the cognitive enhancer aniracetam. An effect similar to that of scopolamine was also found in mice treated with dizocilpine or given an electroconvulsive shock. The findings were interpreted in terms of amnesic and mnesic (and/or antiamnesic) effects of the drugs used. The elevated plus-maze method is simple, not time-consuming, and it does not require manipulation of appetitive or aversive behaviours.

On the basis of these results and a previous study on habituation of exploratory activity in mice (Platel and Porsolt, 1982), we therefore predicted that retention of memory information, as measured by a decrease in transfer latency from the 1st to the 2nd session, attenuates as a function of increasing inter-session intervals, thus suggesting that forgetting occurs over time (Experiment 1). If this hypothesis is valid, drugs which are known to enhance memory in other learning paradigms could improve the poor retention normally observed with long inter-session intervals (Experiment 2). Thus, alaptide and oxiracetam were chosen as reference drugs. Finally in several experimental sets, 21 alaptide analogues were evaluated (Experiment 3).

2. Materials and methods

2.1. Animals

Female mice of the NMRI strain (Konárovice Breeding) weighing 23-30 g were used. They were housed ten per cage and kept in a temperature-regulated (20–22°C) room on a natural photoperiod for at least two weeks before the start of the experiment. Commercial pellet foods and tap water were available ad libitum. All animals were naive to the experimental apparatus. The experiments were conducted between 09:00 and 12:00 h in a room different from the colony room. White noise was used to mask extraneous noises.

The experiments were conducted in agreement with the Ethical Direction of State Law 246/1992(CR) and the Principles of Good Laboratory Practice and Compliance OECD/GD(93)105.

2.2. Drugs

Alaptide (VÚFB 15754), oxiracetam (Medea Research) and the following alaptide analogues (c(Gly-Apr), c(Ala-Apr), c(Gly-Abu), c(Ala-Abu), c(Gly-Ach), c(Gly-Acp)) were dissolved in 0.9% saline solution. The other 15 alaptide analogues were suspended in distilled water. Oxiracetam (60 mg/kg) was administered s.c., and alaptide and its analogues (5 mg/kg) were administered p.o., always in the volume of 0.1 ml/10 g body weight.

2.3. Apparatus

The plus-maze was made from red-and-brown plastic and consisted of two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 16 cm). Open arms had a margin 3 mm high. Enclosed arms were covered with a removable board. The arms extended from a central space (5 × 5 cm). The apparatus was 50 cm above the floor. All arms were cleaned and washed after the mouse was taken out of the apparatus.

2.4. Procedure

The procedure was identical to that described by Itoh et al. (1990). Briefly, the mice were individually placed at the end of one open arm facing away from the central platform. The transfer latency, i.e., the time it took for the mouse to move from the open arm to either of the enclosed arms, was recorded. The criterion of an animal's entry into the enclosed arm was crossing with all four legs of an imaginary line separating the enclosed arm from the central platform. If the mouse did not enter the enclosed arm within 90 s, it was pushed gently on the back into the arm and a transfer latency of 90 s was assigned. After the measurement of transfer latency, the mouse was allowed to move freely in the plus-maze regardless of open and closed arms for 10 s. Then, the mouse was gently taken out of the apparatus and was returned to its home cage.

2.4.1. Experiment 1

The procedure was repeated on day 1 or 4 or 7 or 10 or 14 later. In this retention session, the mouse was again put into the elevated plus-maze and transfer latency was measured. If the animal did not enter the enclosed arm within 90 s, a transfer latency of 90 s was assigned.

2.4.2. Experiment 2

The procedure and the apparatus were identical to those described in the previous experiment. The retention session was performed on the 10th day after the acquisition session. Alaptide or oxiracetam or solvent was administered immediately after the end of the first session.

2.4.3. Experiment 3

The procedure and the apparatus were identical to those described in the previous experiment. Alaptide analogues or solvent was administered immediately after the first session. The experiment consisted of 5 sets with different control groups.

2.5. Statistics

Statistical analysis was performed with the CSS package. Data were processed nonparametrically. The data of mice in which the transfer latency was equivalent to 90 s in both the 1st and the 2nd sessions were not used for the statistical analysis. Thus, 17 out of 594 animals were excluded. (1) To compare transfer latency of animals of independent groups during the first vs. the second session, the Wilcoxon matched-pairs signed-rank test was used. (2) To estimate the effect of the inter-session interval on the transfer latency in Experiment 1, the behavioural data were analyzed using the Kruskal-Wallis analysis of variance (ANOVA) followed by the Conover method (Conover, 1980). The same analysis was used in Experiments 2 and 3 to compare transfer latencies during the first session, i.e., before the treatment. (3) To evaluate the effect of drugs in Experiments 2 and 3, the Mann-Whitney U-test was used.

Table 2
Changes in the transfer latency to the enclosed arm of the elevated plus-maze in mice as a function of inter-session interval

Inter-session interval (day)	n	Transfer latency (s)		Ratio index
		1st session	2nd session	
1	15	42.7 ± 4.5	25.3 ± 3.8 [d]	0.60 ± 0.06
4	15	46.2 ± 6.7	26.2 ± 6.8 [d]	0.62 ± 0.12
7	14	46.7 ± 6.6	32.5 ± 5.3 [b,d]	0.80 ± 0.12
10	13	45.8 ± 5.6	58.8 ± 7.7 [a–c]	1.45 ± 0.25 [a–c]
14	14	48.4 ± 6.4	50.3 ± 6.0 [a–c]	1.27 ± 0.25 [a–c]
		$H = 0.41$	$H = 22.80$	$H = 18.78$
		$P = 0.98$	$P = 0.0001$	$P = 0.0009$

The transfer latency (s) was recorded as described in Section 2.4. The 2nd session was performed on day 1, 4, 7, 10 or 14 after the 1st session. Ratio index means ratio of the transfer latency during the 2nd session to that during the 1st session. Data are expressed as means ± S.E.M. Statistical analysis: Kruskal-Wallis H-test ANOVA (df = 4) followed by comparison among groups according to Conover, $P < 0.05$ [a] vs. day 1, [b] vs. day 4, [c] vs. day 7; Wilcoxon matched-pairs signed-rank test, [d] $P < 0.05$ vs. the 1st session.

With the aim to remove inter- and intra-individual differences in the transfer latency, ratio indexes (calculated for each animal as the ratio of the transfer latency during the second session to that during the first one) were also compared. The criterion for statistical significance was $P < 0.05$.

3. Results

3.1. Experiment 1: effect of inter-session interval on memory retention

Changes in the transfer latency and their dependence on the duration of the inter-session interval are demonstrated in Table 2.

The overall analysis did not reveal a significant difference in the transfer latency during the first session ($H = 0.41$, df = 4, $P = 0.98$) but revealed a significant difference both in the transfer latency during the second session ($H = 22.80$, df = 4, $P = 0.0001$) and in the ratio index ($H = 18.78$, df = 4, $P = 0.0009$). Subsequent comparisons showed that animals retested in the elevated plus-maze on days 1, 4 and 7 after the initial session displayed a significantly shorter transfer latency than those retested on days 10 and 14. The same holds true for comparison of ratio indexes. Further, transfer latencies of animals retested on days 1, 4 and 7 were significantly reduced as compared to those measured during the first session ($Z = 3.41$, $P = 0.0007$ for day 1; $Z = 2.27$, $P = 0.023$ for day 4; $Z = 2.20$, $P = 0.028$ for day 7). In contrast, latencies of mice retested on days 10 and 14 did not differ from those measured during the first session ($Z = 1.43$, $P = 0.16$ for day 10; $Z = 0.31$, $P = 0.75$ for day 14). A significant difference in the transfer latency, but not in the ratio index was found in animals retested on day 7 vs. day 1 or 4.

These results suggest that the mice retested on day 1, 4

Table 3
Effects of alaptide and oxiracetam on the transfer latency to the enclosed arm of the elevated plus-maze in mice

Group	n	Transfer latency (s)		Ratio index
		1st session	2nd session	
Control	19	34.7±3.2	39.5±4.2	1.25±0.15
Alaptide	18	37.7±3.8	24.9±2.3 [a,b]	0.73±0.07 [b]
Oxiracetam	19	38.7±3.6	18.9±1.7 [a,b]	0.57±0.07 [b]

The transfer latency (s) was recorded as described in Section 2.4. Alaptide (5 mg/kg) and oxiracetam (60 mg/kg) were administered p.o. and s.c., respectively, immediately after the 1st session. The 2nd session was performed on day 10 after the 1st session. Ratio index, ratio of the transfer latency during the 2nd session to that during the 1st session. Data are expressed as mean±S.E.M. values. Statistical analysis: Wilcoxon matched-pairs signed-rank test, [a] $P < 0.05$ vs. the 1st session; Mann-Whitney U-test, [b] $P < 0.05$ vs. control group.

or 7 escaped from the open arm into the enclosed arm in significantly shorter time than those retested on day 10 or 14. This may be interpreted as an ability of animals to remember a safe place, i.e., the dark area of the elevated plus-maze. In contrast, the transfer latencies of animals retested on day 10 or 14 show that the memory trace is forgotten over time. On the basis of these results, a 10-day inter-session interval was chosen for testing drugs which were expected to enhance memory.

3.2. Experiment 2: effect of alaptide and oxiracetam on memory retention

The results obtained with two memory-enhancing drugs are shown in Table 3.

There was no significant difference in the transfer latency during the 1st session among of animals divided in random in the three groups (Kruskal-Wallis test: $H = 0.97$, df = 2, $P = 0.61$). In control animals, the transfer latency during the retention session corresponded to that measured during the initial one ($Z = 1.00$, $P = 0.32$). In contrast, with alaptide and oxiracetam treatment the transfer latency was found significantly decreased in the retention session compared to the first session ($Z = 3.08$, $P = 0.002$ for alaptide and $Z = 3.58$, $P = 0.0003$ for oxiracetam). There was a significant difference both in the transfer latency and in the ratio index between the control and alaptide-treated groups ($Z = 2.43$, $P = 0.015$ and $Z = 2.67$, $P = 0.0075$, respectively) as well as between the control and oxiracetam-treated groups ($Z = 3.47$, $P = 0.0005$ and $Z = 3.81$, $P = 0.0001$, respectively). These results suggest, therefore, that both alaptide and oxiracetam enhanced retention of information received during the first session, as predicted.

3.3. Experiment 3: effect of alaptide analogues on memory retention

The effects of alaptide analogues on the transfer latency in mice are presented in Table 4. Statistical evaluation of the effects is summarized in detail in Table 5.

There was no significant difference in the transfer latency measured during the 1st session (i.e., before administration of the drugs) among independent groups within pertinent sets (Kruskal-Wallis test: $H = 5.65$, df = 6, $P = 0.47$ for Set 1; $H = 3.26$, df = 6, $P = 0.78$ for Set 2; $H = 7.45$, df = 4, $P = 0.11$ for Set 3; $H = 0.11$, df = 2, $P = 0.95$ for Set 4; $H = 0.76$, df = 3, $P = 0.86$ for Set 5). In all control groups, the transfer latency during the 2nd session did not significantly differ from that during the 1st one. In contrast, a significant reduction in the transfer latency during the 2nd session as compared to that during the 1st one was found with 13 alaptide analogues. When compared with the corresponding control group, a significant shortening of transfer latency during the 2nd session was found with 7 alaptide analogues: c(Ala-Abu), c(Gly-Acp), c(3-MeAch-Gly), c(3-MeAch-D-Ser), c(3-MeAch-L-Val), c(3-MeAch-D-Val) and c(Ala-Achpt). Further, a significantly decreased ratio index was found with 6 alaptide analogues: c(Ala-Abu), c(3-MeAch-Gly), c(3-MeAch-L-Ser), c(3-MeAch-D-Ser), c(3-MeAch-Gly), c(Ala-Achpt).

Table 4
Effect of alaptide analogues on the transfer latency to the enclosed arm of the elevated plus-maze in mice

Set	Group	n	Transfer latency (s)		Ratio index
			1st session	2nd session	
1	Control	20	43.6±5.0	38.8±6.2	1.04±0.20
	c(Gly-Apr)	16	41.9±4.9	39.3±6.8	1.00±0.17
	c(Ala-Apr)	16	50.8±5.2	30.4±4.2 [a]	0.66±0.09
	c(Gly-Abu)	17	36.5±5.4	29.9±5.3	0.96±0.15
	c(Ala-Abu)	15	46.2±5.9	18.2±2.8 [a,b]	0.47±0.08 [b]
	c(Gly-Ach)	15	47.9±6.5	32.0±7.3	0.75±0.15
	c(Gly-Acp)	16	42.2±5.3	24.6±6.3 [a,b]	0.71±0.23
2	Control	19	51.6±4.9	39.8±6.4	0.80±0.12
	c(3-MeAch-D-Ala)	15	55.1±4.7	29.6±6.3 [a]	0.61±0.14
	c(3-MeAch-Gly)	17	48.7±4.8	21.8±2.2 [a,b]	0.53±0.09 [b]
	c(3-MeAch-L-Ser)	16	61.4±5.6	27.6±4.8 [a]	0.47±0.08 [b]
	c(3-MeAch-D-Ser)	17	55.1±4.2	23.3±2.9 [a,b]	0.45±0.07 [b]
	c(3-MeAch-L-Val)	17	50.8±6.0	22.9±4.7 [a,b]	0.53±0.08
	c(3-MeAch-D-Val)	15	51.3±5.4	18.7±2.3 [a,b]	0.41±0.05 [b]
3	Control	18	35.7±4.2	41.4±5.0	1.24±0.16
	c(3-MeAch-D-Phe)	16	39.1±4.9	31.8±4.6	0.96±0.17
	c(3-MeAch-L-Phe)	19	36.7±3.1	35.4±4.3	1.03±0.13
	c(L-Ala-2-MeAcp)	18	41.7±3.4	40.2±3.9	1.05±0.12
	c(Ach-L-Ala)	16	30.9±1.9	30.3±2.4	1.04±0.10
4	Control	19	35.5±4.3	34.6±6.7	0.97±0.12
	c(Ala-Achpt)	18	39.2±6.0	19.1±3.7 [a,b]	0.76±0.26 [b]
	c(3-MeAch-D-Leu)	19	39.3±5.7	25.1±4.4	0.7±0.19
5	Control	18	40.0±3.5	36.5±7.6	0.89±0.15
	c(4-MeAch-Gly	18	37.8±3.4	23.3±4.7 [a]	0.63±0.11
	c(4-*t*BuAch-L-Ala)	20	40.4±5.0	18.4±2.8 [a]	0.64±0.13
	c(4-*t*BuAch-Gly)	19	41.8±4.1	26.9±4.7 [a]	0.71±0.14

The transfer latency (s) was recorded as described in Section 2.4. Alaptide analogues (always 5 mg/kg) were administered p.o. immediately after the 1st session. The 2nd session was performed on day 10 after the 1st session. Ratio index, ratio of the transfer latency during the 2nd session to that during the 1st session. Data are expressed as mean±S.E.M. values. Statistical analysis: Wilcoxon matched-pairs signed-rank test, [a] $P < 0.05$ vs. the 1st session; Mann-Whitney U-test, [b] $P < 0.05$ vs. the corresponding control group (for details see Table 5).

Table 5
Statistical analysis of the effects of alaptide analogues as demonstrated in Table 4

Set	Group	Wilcoxon test		Mann-Whitney test			
		TL		TL		RI	
		1st vs. 2nd session		2nd session		2nd/1st session	
		Z	P	Z	P	Z	P
1	Control	0.952	0.343				
	c(Gly-Apr)	1.086	0.280	0.175	0.862	0.223	0.823
	c(Ala-Apr)	3.025	**0.002**	0.525	0.601	1.003	0.318
	c(Gly-Abu)	1.065	0.289	1.204	0.231	0.076	0.942
	c(Ala-Abu)	3.351	**0.0008**	2.650	**0.008**	2.367	**0.018**
	c(Gly-Ach)	1.448	0.150	1.100	0.274	1.067	0.288
	c(Gly-Acp)	2.741	**0.006**	2.213	**0.027**	1.942	0.052
2	Control	1.670	0.098				
	c(3-MeAch-D-Ala)	2.272	**0.023**	1.648	0.102	1.890	0.058
	c(3-MeAch-Gly)	3.361	**0.0008**	2.424	**0.015**	2.139	**0.032**
	c(3-MeAch-L-Ser)	3.232	**0.001**	1.589	0.115	2.699	**0.007**
	c(3-MeAch-D-Ser)	3.550	**0.0004**	2.186	**0.029**	2.773	**0.005**
	c(3-MeAch-L-Val)	3.148	**0.002**	2.709	**0.007**	1.870	0.061
	c(3-MeAch-D-Val)	3.408	**0.0006**	2.844	**0.004**	2.671	**0.007**
3	Control	0.327	0.744				
	c(3-MeAch-D-Phe)	1.138	0.258	1.501	0.136	1.518	0.132
	c(3-MeAch-L-Phe)	0.201	0.840	0.927	0.356	1.064	0.290
	c(L-Ala-2-MeAcp)	0.071	0.942	0.142	0.888	0.554	0.582
	c(Ach-L-Ala)	0.063	0.952	1.328	0.186	0.449	0.654
4	Control	0.101	0.918				
	c(Ala-Achpt)	2.940	**0.003**	2.036	**0.042**	2.294	**0.022**
	c(3-MeAch-D-Leu)	1.952	0.051	0.715	0.476	1.489	0.139
5	Control	0.544	0.587				
	c(4-MeAch-Gly	2.414	**0.016**	1.234	0.220	0.902	0.369
	c(4-*t*BuAch-L-Ala)	2.744	**0.006**	1.915	0.055	1.579	0.117
	c(4-*t*BuAch-Gly)	2.113	**0.035**	0.972	0.333	0.805	0.422

Wilcoxon matched-pairs signed-rank test was used to compare the transfer latency (TL) during the 1st session to that during the 2nd session in a given control or with an alaptide analogue-treated group. Mann-Whitney *U*-test was used to compare the effect of a given alaptide analogue with the corresponding control group with respect to both the transfer latency (TL) in the 2nd session and the ratio index (RI). Significant values are indicated in bold-faced types. Absolute mean ± S.E.M. values are summarized in Table 4.

Thus, with 5 alaptide analogues, c(Ala-Abu), c(Ala-Achpt), c(3-MeAch-Gly), c(3-MeAch-D-Ser), and c(3-MeAch-D-Val), both the transfer latency during the 2nd session and the ratio index were significantly changed. In c(Gly-Acp), a significant reduction of transfer latency was evident, but the change in ratio index was not statistically significant.

4. Discussion

Among the behavioural procedures available for research into learning and memory and for identifying nootropic drugs, the habituation of exploratory activity in the elevated plus-maze may be useful as an experimental model. The present experiments confirmed that the time it took for the mice to escape from the open arm to the enclosed arm (so called 'transfer latency') was significantly shortened when the two sessions were separated by 24 h and that the time lengthened with increasing inter-session intervals, suggesting that forgetting of information was forgotten over time. Thus, there is an agreement with findings of Itoh et al. (1990, 1991) that transfer latency may be shortened when mice can remember the location of the enclosed arm. Moreover, the present results demonstrated no memory retention in animals subjected to 10- and 14-day inter-session intervals. In fact, this finding is consistent with the data on the habituation potency of mice reported by Platel and Porsolt (1982). In the experimental set-up of these authors, the differences in memory performance with a 7-day inter-session interval failed to reach significance. In our study, memory retention disappeared approximately 3 days later, e.g., at the 10th day. This difference may result from the different complexity of the experimental devices used.

We used the criterion of a significantly reduced transfer latency during the 2nd session and a significantly reduced ratio index to decide on the beneficial effect of a given drug. Thus, the poor retention of information observed with a 10-day inter-session interval was enhanced by post-training administration of oxiracetam and alaptide. This effect is in accord with the well-known beneficial effects of oxiracetam on learning and memory processes in animals and humans (Banfi and Dorigotti, 1984; Merlini and Pinza, 1989; Nicholson, 1990) and those of alaptide in

animals (see Section 1). Whereas the present results allow us to exclude a direct effect of alaptide or oxiracetam on memory processes during the acquisition session, we cannot decide whether the consolidation or retrieval phase of the memorization process was affected. In general, both drugs participate in mechanisms modulating long-term memory retention. Further research in this context is needed.

Of particular interest was the finding that retention of information on the spatial arrangement of the 4-arm maze was enhanced by several alaptide analogues. Both the criterion of a significantly reduced transfer latency during the 2nd session and the criterion on significantly reduced ratio index were fulfilled after post-training administration of the following substances: c(Ala-Abu), c(Ala-Achpt), c(3-MeAch-Gly), c(3-MeAch-D-Ser), c(3-MeAch-D-Val). In fact, the efficiency of all these substances corresponded to that in animals treated with alaptide or oxiracetam. Although alaptide as well as its synthetized compounds is closely related to a peptide naturally occurring in the brain, the mechanism of action remains unknown. Alaptide moderately influences the dopaminergic neuronal system, however, there is no evidence for a direct action on dopamine receptors (Valchář et al., 1985). The mechanism of action of alaptide seems to be different from that of classical nootropics. Nevertheless, the present results support the therapeutic value of alaptide and its analogues for clinical practice.

Previous tests of pharmacological and biological activities have already confirmed the optimal structure of the spirocyclic dipeptide alaptide (VÚFB 15754), i.e., cyclo(L-alanyl-1-amino-1-cyclopentanecarbonyl). The spirocyclic dipeptides investigated in this study are similar to other structural analogues used to define the optimal structure of alaptide (Vanžura et al., 1986; Vinšová et al., 1993, 1994).

The target design was to model the size of the cycloalkane ring of the cyclic dipeptide from the smallest member C_3 to C_7, i.e., the series of cyclopropane, cyclobutane, cyclopentane, cyclohexane and cycloheptane. The second amino acid component was either L-alanine or glycine. In this series, the importance of the structure of alaptide was confirmed again, which underlines the dominant position of the cyclopentane ring. A decreased activity was observed with the cyclobutane ring; adequate activities were shown by the peptides with cyclohexane or cycloheptane rings. Cyclopropane derivatives were quite inactive irrespective of whether glycine or alanine was the second amino acid residue. Generally, the active structures preferred L-alanine to glycine in this position.

Isomers in the cyclohexane series in which methyl or *tert*-butyl was substituted in positions 2–4 followed the optimal bonding conformation. Combinations with an optical isomer of the second amino acid should maximally strengthen the ideal conformation. Of the series above, position 3 is favoured more than position 4 or 2. The nature of the second amino acid component had little effect, and the optical configuration had no effect.

From the physical point of view, our spirocyclic dipeptide compounds are poorly soluble or totally insoluble in water, thus it is very difficult to evaluate the series pharmacologically. We tested the compounds in suspension. We intend to increase the solubility of prospective dipeptides by means of synthesis of *N*-prolonged peptides as prodrugs. Essentially, this means constructing linear precursors with active amino acid sequences that would be enzymatically hydrolysed and than undergo cyclization to form the spirocyclic dipeptide in the organism, as we have published earlier (Kasafírek et al., 1992).

From the neurobehavioural point of view, the present results can be summarised as follows. Several substances derived from the alaptide molecule prolonged memory retention in female mice. The improvement of memory performance measured by means of the transfer latency from the open arm to enclosed arm with a 10-day inter-session interval was similar to that seen in alaptide or oxiracetam treated animals. The effect of selected alaptide analogues will be further investigated using other behavioural paradigms and in relation to the impairments of memory caused by manipulation of different neurotransmitter systems.

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