Physiology and Behavior, Vol. 13, pp. 381–388. Brain Research Publications Inc., 1974. Printed in the U.S.A.

The Effects of ACTH- and Vasopressin-Analogues on CO_2-induced Retrograde Amnesia in Rats

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(Received 4 February 1974)

RIGTER, H., H. VAN RIEZEN AND D. DE WIED. *The effects of ACTH- and vasopressin-analogues on CO_2-induced retrograde amnesia in rats.* PHYSIOL. BEHAV. 13(3) 381–388, 1974. – Amnesia for a one-trial step-through passive avoidance response was induced in rats by application of CO_2 until respiratory arrest occurred. The ACTH-analogue $ACTH_{4-10}$ alleviated the amnesia when administered 1 hr prior to the retrieval test but not when given 1 hr prior to the acquisition trial. The behaviourally inert ACTH-analogue $ACTH_{11-24}$ appeared to have no effect on the amnesia. The vasopressin-analogue desglycinamide lysine vasopressin (DG-LVP) antagonized the amnesia when administered 1 hr prior to the acquisition trial or 1 hr prior to the test trial. The relevance of these date to present theories on amnesia is discussed.

$ACTH_{4-10}$ Amnesia Desglycinamide lysine vasopressin CO_2 Memory consolidation Memory retrieval

FLEXNER and Flexner [6] reported that amnesia in mice induced by the intracerebral injection of puromycin was almost completely reversed when the animals were treated with an ACTH-preparation before or within 16 hr of the acquisition of an avoidance response. In a later investigation from the same laboratory [9], in which a chemically pure ACTH-preparation was used, no anti-amnesic effect could be detected; the originally observed anti-amnesic effect of ACTH was attributed to a vasopressin impurity. This assumption was validated by the finding that desglycinamine lysine vasopressin (DG-LVP) caused a reduction of the amnesia under the same circumstances as the crude ACTH-preparation used by Flexner and Flexner [6]. DG-LVP almost completely lacks the endocrine effects of vasopressin but exerts the same effects on learning behaviour in rats [25]. These results do not exclude an effect of ACTH on amnesia. Firstly, puromycin like other inhibitors of protein synthesis affects the function of the pituitary-adrenal axis [16], thereby possibly contaminating the effects of administration of exogeneous ACTH. Secondly, Flexner and Flexner [6] and Lande, Flexner and Flexner [9] administered ACTH prior to the amnesic treatment but not prior to the retrieval test. Furthermore, these investigators used ACTH, which apart from exerting an effect on the central nervous system also stimulates the production of corticosteroids from the adrenals. These corticosteroids may exert effects on behaviour which are opposite to those of ACTH [3,21]. Moreover, surgical removal of the adrenals, which causes increased ACTH release, results in a reduction of amnesia [5]. Although it is possible that adrenalectomy also causes an increased release of vasopressin [17], further studies on the possible anti-amnesic effect on ACTH seem justified. In the present investigation we compared the anti-amnesic effects of the ACTH-analogue $ACTH_{4-10}$ with the effects of DG-LVP. $ACTH_{4-10}$ lacks virtually all corticotrophic activities but possesses the same effects with respect to acquisition and extinction of avoidance behaviour as the parent hormone ACTH [22]. CO_2 was used as the amnesic agent. The efficacy of CO_2 as an amnesic agent compares favourably with that of the frequently used electroconvulsive shock [13,14].

METHOD

In each experiment 120 male rats of an inbred Wistar derived strain were used. They were obtained from TNO-Zeist, the Netherlands. The rats were 9–10 weeks of age

and weighed 230–260 g at the start of the experiments. The animals were housed 10 per cage (50 × 35 × 20 cm) with ad lib access to water and standard food pellets.

The step-through passive avoidance apparatus described by Ader, Weijnen and Moleman [1] was used. This consisted of a 40 × 40 × 40 cm Lucite chamber with black walls and a grid floor. The front wall was situated at the edge of a table. A 6 cm wide, 25 cm long runway protruded from the front wall over this edge. The runway was brightly lit by a 40 W lamp positioned 40 cm above it while the chamber was in darkness. When placed on the runway an animal could enter the chamber through a 6 × 6 cm opening in the front wall which could be closed by a hand-operated guillotine door. A scrambled foot shock could be delivered through the grid floor of the chamber. Shock was produced by a 500 V a.c. source through a variable resistance (0.5–5.5 MΩ). The resistance was adjusted to yield a shock intensity of 0.50 mA. The duration of the shock was controlled automatically and set for 3 sec.

The animals were randomly divided into 12 groups of 10. They were given 3 pretraining trials on Day 1 of the experiment. A pretraining trial consisted of placing the rat at the end of the runway while facing the open entrance. The latency of the animal to enter the chamber was recorded in tenths of a second. Upon entering the chamber with all 4 feet, the door was closed and the animal was left in the chamber for 10 sec. The interval between pretraining trials was approximately 2 hr. On Day 2 a single acquisition trial was run. It was identical to a pretraining trial except that at the end of it a foot shock was given to 8 groups of animals (FS groups). The remaining 4 groups were left undisturbed in the chamber for another 3 sec (No FS groups). Immediately on termination of the acquisition trial the rats were either subjected to amnesic treatment (CO_2 groups) or to sham amnesic treatment (No CO_2 groups). A 50 × 35 × 20 cm black plastic box with transparent cover was filled with CO_2 until oxygen measurements by means of a Teledyne Analytical Instruments Model 330 D Percent Oxygen Detector yielded zero readings. The rat was placed in the box until respiratory arrest occurred and then revived by artificial respiration. Following revival it was returned to its home cage. Pilot experiments indicated that it usually took 30–35 sec for respiratory arrest to occur. Therefore, rats receiving sham treatment were placed in an identical but air-filled box for 35 sec before being returned to their cages.

Twenty-four hr after acquisition a single test trial was run. When a rat did not enter the chamber within 300.0 sec, it was taken from the runway and a score of 300.0 sec was arbitrarily assigned to it.

The ACTH-analogues were dissolved in a phosphoric acid solution of pH 3.5. This solution was diluted with saline to a concentration of 100 μg/ml. It was neutralized to pH 7 with sodium bicarbonate before injection. DG-LVP was dissolved in a hydrochloric acid solution of pH 3.5. This solution was diluted with saline to a concentration of 10 μg/ml and subsequently neutralized with sodium bicarbonate to pH 7 prior to injection. Either 1 ml saline or 1 ml drug solution was injected s.c. 1 hr prior to the acquisition trial and/or the test trial. The experimental design is given in Table 1.

The results were analysed with the Yates test [26]. The test scores were divided into 3 classes: (1) latencies of 0–10.0 sec; (2) latencies of 10.1–299.9 sec; (3) scores of 300.0 sec. Rats entering the chamber within 10.0 sec were considered to have no passive avoidance tendency. Rats entering within 10.1–299.9 sec were regarded to display an incomplete passive avoidance tendency. A refusal to enter within 300.0 sec was considered to be a complete avoidance response. In the analysis of the results the three classes received a statistical weighting of 0, 1 and 2 respectively.

TABLE 1

DESIGN OF EXPERIMENTS 1, 2 AND 3

Group	Foot Shock	CO_2	Treatment 1 hr prior to acquisition	test
No FS–No CO_2	–	–	saline	saline
No FS–No CO_2	–	–	saline	drug
No FS–CO_2	–	+	saline	saline
No FS–CO_2	–	+	saline	drug
FS–CO_2	+	+	saline	saline
FS–CO_2	+	+	drug	saline
FS–CO_2	+	+	saline	drug
FS–CO_2	+	+	drug	drug
FS–No CO_2	+	–	saline	saline
FS–No CO_2	+	–	drug	saline
FS–No CO_2	+	–	saline	drug
FS–No CO_2	+	–	drug	drug

FS = foot shock; No FS = no foot shock. CO_2 = CO_2-treatment; No CO_2 = no CO_2-treatment.

EXPERIMENT 1

The effect of $ACTH_{4\text{-}10}$ on CO_2-induced amnesia for the one-trial passive avoidance response was studied. $ACTH_{4\text{-}10}$ was administered s.c. 1 hr prior to acquisition and/or retrieval test. The dose used was 100 μg/rat.

Results

Pretraining of the step-through response resulted in short latencies at the acquisition trial. Pretreatment with $ACTH_{4\text{-}10}$ did not affect the latencies at this trial (Table 2, part A). The No FS rats maintained short latencies at the test trial, irrespective of the pretreatment. No significant differences could be detected between No FS-No CO_2 and No FS-CO_2 groups (Table 2, part B). $ACTH_{4\text{-}10}$ did not affect passive avoidance behaviour in FS-No CO_2 groups, whether injected before the acquisition trial, the test trial, or before both trials (compared to the placebo FS-No CO_2 group: $z = 0.63$, 0 and 0.63, respectively) (Fig. 1).

TABLE 2

ACQUISITION AND TEST TRIAL STEP-THROUGH LATENCIES OF NON-SHOCKED GROUPS OF RATS FOLLOWING TREATMENT WITH $ACTH_{4-10}$

A Acquisition Trial				B Test Trial			
group	number of rats	drug treatment	latency*† (sec)	group	number of rats	drug treatment	latency*† (sec)
pooled	80	saline	1.5 ± 0.2	No FS–No CO_2	10	saline	1.8 ± 0.2
				No FS–No CO_2	10	$ACTH_{4-10}$	1.7 ± 0.1
pooled	40	$ACTH_{4-10}$	1.6 ± 0.2	No FS–CO_2	10	saline	1.6 ± 0.2
				No FS–CO_2	10	$ACTH_{4-10}$	1.6 ± 0.2

*Mean ± standard error of the mean
†Differences between the groups in the column are not significant (two-tailed Mann-Whitney U test).
No FS–No CO_2: no foot shock, no CO_2-treatment
No FS–CO_2: no foot shock, CO_2-treatment
Saline: 1 ml saline s.c. 1 hr prior to trial
$ACTH_{4-10}$: 100 μg $ACTH_{4-10}$/rat s.c. 1 hr prior to trial

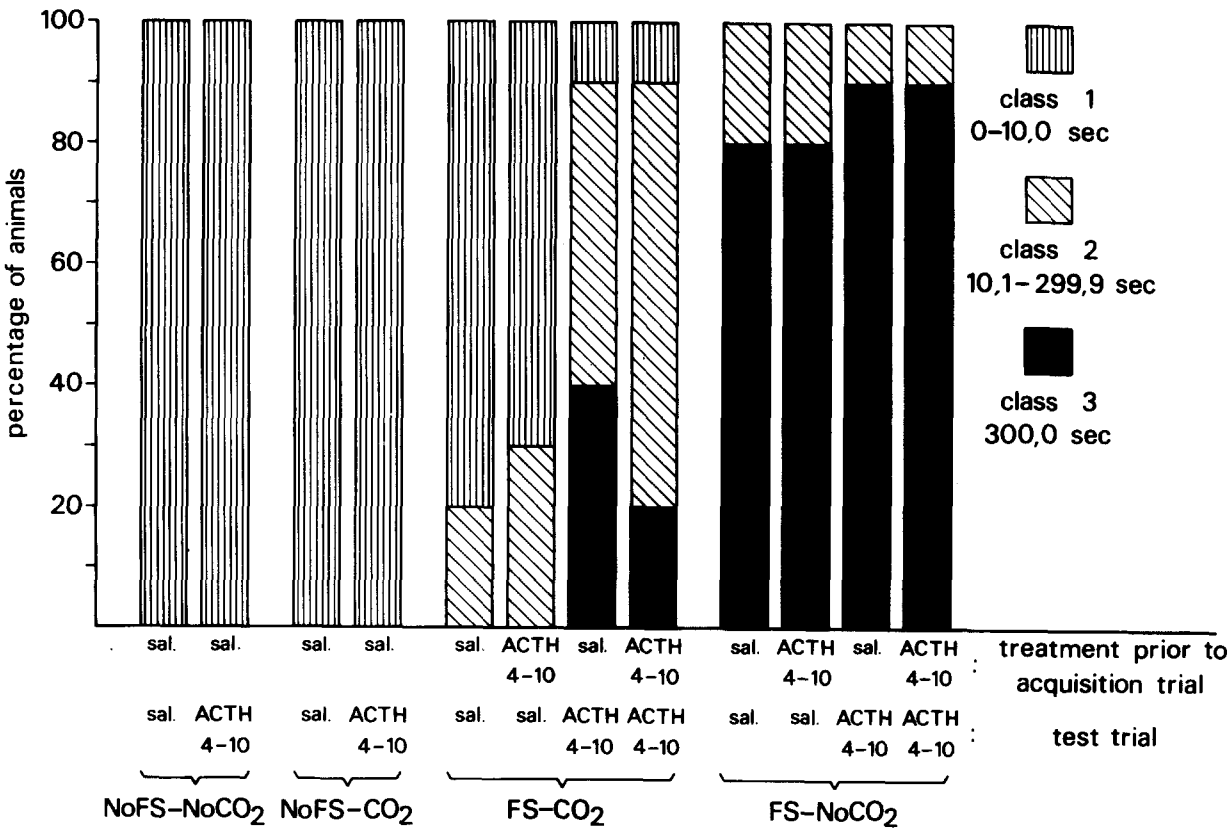

FIG. 1. The effect of $ACTH_{4-10}$ on CO_2-induced amnesia for a passive avoidance response. The figure presents the latencies at the test trial. The scores were divided into 3 classes: (1) 0–10.0 sec (no avoidance); (2) 10.1-299.9 sec (non-optimal avoidance); and (3) 300.0 sec (optimal avoidance). Saline: 1 ml saline/rat s.c. 1 hr prior to trial; $ACTH_{4-10}$: 100 μg $ACTH_{4-10}$/rat s.c. 1 hr prior to trial. FS: foot shock; No FS: no foot shock; CO_2: CO_2-treatment; No CO_2: No CO_2.

CO_2 was able to induce amnesia. This was apparent from the test scores of the placebo FS-CO_2 group. Only 2 out of 10 animals of this group had a latency longer than 10.0 sec. The scores of the other 8 rats were within the range of performance of the No FS groups.

Accordingly, the difference between the placebo FS-CO_2 and No FS-CO_2 groups was not significant (in both cases: $z = 1.49$, $p>0.05$). On the other hand, the difference between the placebo FS-CO_2 and the placebo FS-No CO_2 groups was significant ($z = 4.00$, $p<0.0001$) (Fig. 1).

When administered prior to the acquisition trial, $ACTH_{4-10}$ had no effect on the amnesia ($z = 0.52$, not significant). However, treatment with $ACTH_{4-10}$ 1 hr before the test trial resulted in a significant reduction of the amnesia. This was true for the group which received $ACTH_{4-10}$ before the test trial as well as for the group which received the peptide before both the acquisition and the test trial (compared to the placebo FS-CO_2 group: $z = 3.21$, $p<0.001$; and $z = 3.07$, $p<0.01$, respectively). However, the reduction of the amnesia due to pre-test treatment with $ACTH_{4-10}$ was not complete as comparisons with similarly treated FS-No CO_2 groups yielded significant differences ($z = 2.30$, $p<0.05$; and $z = 3.02$, $p<0.001$, for groups treated with $ACTH_{4-10}$ prior to the test trial and groups treated with $ACTH_{4-10}$ prior to both the acquisition and the test trial, respectively) (Fig. 1).

EXPERIMENT 2

Greven and de Wied [8] demonstrated that the effects of ACTH on acquisition and extinction of avoidance behaviour could be replicated by administration of various C terminal ACTH-analogues like $ACTH_{1-10}$ and $ACTH_{4-10}$. The sequence $ACTH_{11-24}$, however, was ineffective. The following experiment was designed to study whether $ACTH_{11-24}$ affected the CO_2-induced amnesia for the step-through passive avoidance response. $ACTH_{11-24}$ was injected in a dose of 100 μg/rat s.c.

Results

Administration of $ACTH_{11-24}$ did not alter the step-through latencies at the acquisition trial (Table 3, part A). Similarly, $ACTH_{11-24}$ did not affect the latencies of No FS rats at the test trial (Table 3, part B).

The peptide had no detectable influence on passive avoidance behaviour in FS-No CO_2 groups, whether injected before the acquisition trial, the test trial, or before both trials (compared to the placebo FS-No CO_2 group $z = 0.52$, 0 and 0, respectively) (Fig. 2).

The CO_2-treatment resulted in amnesia in the placebo FS-CO_2 group (compared to the placebo FS-No CO_2 group: $z = 4.00$; $p<0.0001$). The amnesia in this group was almost complete: the differences with the placebo No FS-No CO_2 and No FS-Co_2 groups were not significant (in both cases: $z = 1.49$).

Amnesia was unaffected by $ACTH_{11-24}$, whether injected before the acquisition trial, the test trial, or before both trials (compared to the placebo FS-CO_2 group: $z = 0.63$, 0.52 and 0.52, respectively, not significant; compared to the similarly treated FS-No CO_2 groups: $z = 4.02$, $p<0.0001$; $z = 3.88$, $p<0.0001$; and $z = 3.88$, $p<0.0001$, respectively). The $ACTH_{11-24}$-treated FS-CO_2 groups did not differ significantly from each other ($z \leq 1.12$).

EXPERIMENT 3

DG-LVP has an anti-amnesic effect when administered prior to the acquisition of an avoidance response [9]. This is an effect which is qualitatively different to what was found in Experiment 1 for $ACTH_{4-10}$. This observation is in accordance with the general finding that vasopressin- and ACTH-analogues have different behavioural effects. Vasopressin exerts a long term effect on the extinction of active avoidance responses while the effect of $ACTH_{4-10}$ is of a short term nature [23,24]. Similarly, a single injection of DG-LVP results in a long term enhancement of passive

TABLE 3

ACQUISITION AND TEST TRIAL STEP-THROUGH LATENCIES OF NON-SHOCKED GROUPS OF RATS FOLLOWING TREATMENT WITH $ACTH_{11-24}$

A Acquisition Trial				B Test Trial			
group	number of rats	drug treatment	latency*† (sec)	group	number of rats	drug treatment	latency*† (sec)
pooled	80	saline	1.8 ± 0.2	No FS–No CO_2	10	saline	1.6 ± 0.1
				No FS–No CO_2	10	$ACTH_{11-24}$	1.7 ± 0.2
pooled	40	$ACTH_{11-24}$	1.7 ± 0.1	No FS–CO_2	10	saline	1.6 ± 0.2
				No FS–CO_2	10	$ACTH_{11-24}$	1.6 ± 0.2

*Mean ± standard error of the mean
†Differences between the groups in the column are not significant (two-tailed Mann-Whitney U test).
$ACTH_{11-24}$: 100 μg $ACTH_{11-24}$/rat s.c. 1 hr prior to trial
See further the legend to Table 1.

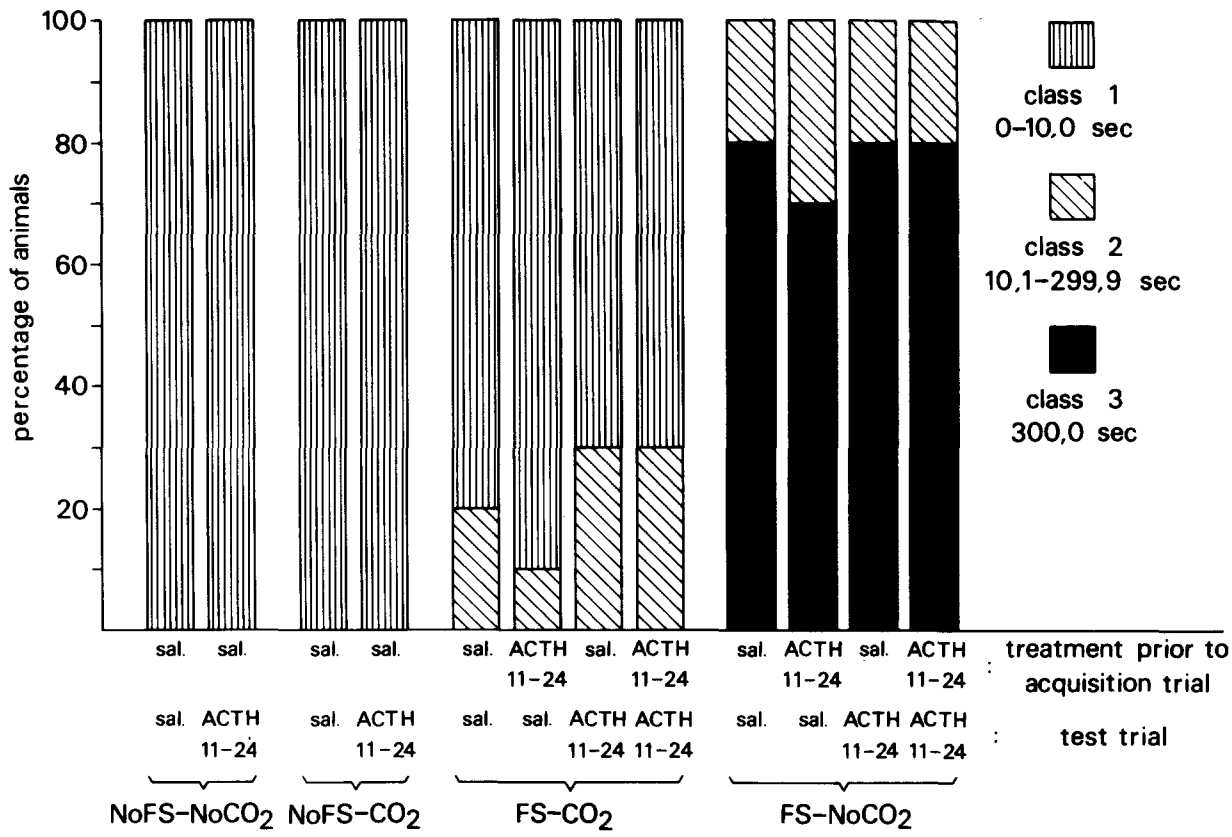

FIG. 2. The effect of $ACTH_{11-24}$ on CO_2-induced amnesia for a passive avoidance response. Saline: 1 ml saline/rat s.c. 1 hr prior to trial; $ACTH_{11-24}$: 100 μg $ACTH_{11-24}$/rat s.c. 1 hr prior to trial. See further the legend to Fig. 1.

TABLE 4

ACQUISITION AND TEST TRIAL LATENCIES OF NON-SHOCKED GROUPS OF RATS FOLLOWING TREATMENT WITH DESGLYCINAMIDE LYSINE VASOPRESSIN

A Acquisition Trial				B Test Trial			
group	number of rats	drug treatment	latency*† (sec)	group	number of rats	drug treatment	latency*† (sec)
pooled	80	saline	1.7 ± 0.1	No FS–NO CO_2	10	saline	1.8 ± 0.2
				No FS–No CO_2	10	DG–LVP	1.6 ± 0.2
pooled	40	DG–LVP	1.8 ± 0.1	No FS–CO_2	10	saline	1.7 ± 0.1
				No FS–CO_2	10	DG–LVP	1.7 ± 0.2

*Mean ± standard error of the mean
†Differences between the groups in the column are not significant (two-tailed Mann-Whitney U test).
DG–LVP: 10 μg desglycinamide lysine vasopressin/rat s.c. 1 hr prior to trial
See further the legend to Table 1.

avoidance behaviour whereas $ACTH_{4-10}$ only causes a temporary enhancement of this behaviour [19]. In the following experiment the effect of DG-LVP on CO_2-induced amnesia for the step-through passive avoidance response was studied. DG-LVP was administered in a dose of 10 μg/rat s.c.

Results

Pretreatment with DG-LVP had no effect on the step-through latencies during the acquisition trial (Table 4, part A). Similarly, DG-LVP did not affect the latencies of No FS animals during the test trial (Table 4, part B).

Passive avoidance of animals from FS-No CO_2 groups was unaffected by DG-LVP, whether injected before the acquisition trial, the test trial or before both trials (compared to the placebo FS-No CO_2 group: $z = 0.52$ in all cases; not significant).

CO_2 induced amnesia in the placebo FS-CO_2 group (compared to the placebo FS-No CO_2 group: $z = 4.13$, $p<0.0001$). Amnesia in this group was almost complete: the difference to the No FS-No CO_2 and No FS-CO_2 groups was not significant (in both cases: $z = 1.02$) (Fig. 3).

DG-LVP led to a reduction of amnesia when administered 1 hr prior to the acquisition trial (compared to the placebo FS-CO_2 group: $z = 3.04$, $p<0.01$). In addition, DG-LVP caused a reversal of amnesia when injected 1 hr prior to the test trial (compared to the placebo FS-CO_2 group: $z = 2.30$, $p<0.05$).

Administration of DG-LVP prior to both the acquisition and the test trial also resulted in a reduction of the amnesia (compared to the placebo FS-CO_2 group: $z = 2.65$, $p<0.01$). The DG-LVP-treated FS-CO_2 groups did not differ significantly from each other ($z \leq 1.13$) (Fig. 3). The reduction of the amnesia in the DG-LVP-treated FS-CO_2 groups was not complete as comparisons with similarly treated FS-No CO_2 groups yielded significant differences ($z = 2.02$, $p<0.05$ for the group which received DG-LVP prior to the acquisition trial; $z = 2.99$, $p<0.01$ for the group which received DG-LVP prior to the test trial; and $z = 2.88$, $p<0.01$ for both the group which was treated with the peptide prior to both trials).

DISCUSSION

Amnesia can be antagonized by pharmacological treatments. The following compounds, for example, have been reported to reduce amnesia: amitriptyline [4]; Piracetam [7]; pentylenetetrazol [20] and strychnine [11]; α-methyl-para-tyrosine, propranolol and chlorpromazine [10]. This group of compounds is so pharmacologically heterogeneous that a uniform mechanism of action seems to be excluded. Most of these investigations offer insufficient guarantees that the anti-amnesic effect of these compounds has not been brought about in a non-specific way. For, in general, these investigations made use of only one type of behavioural task (avoidance tasks), and of only one type of amnesic agent (electroconvulsive shock).

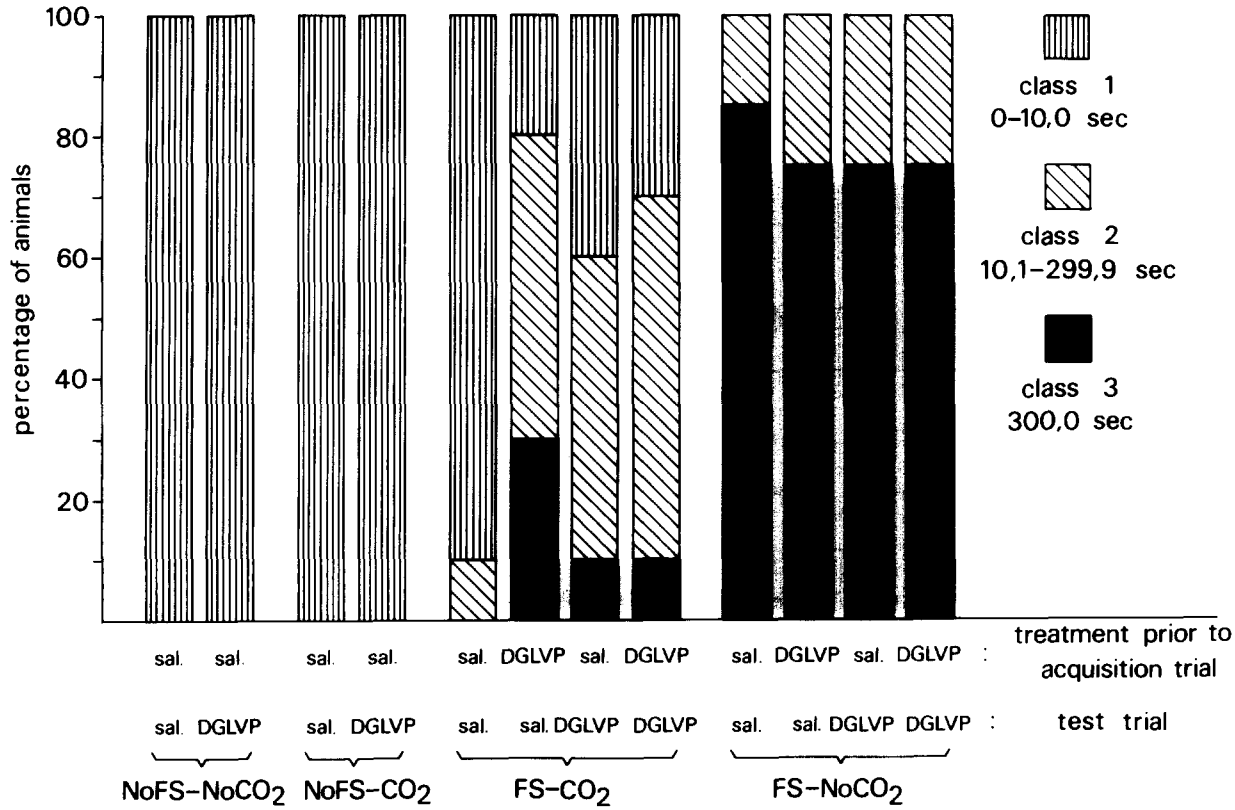

FIG. 3. The effect of desglycinamide lysine vasopressin on CO_2-induced amnesia for a passive avoidance response. Saline: 1 ml saline/rat s.c. 1 hr prior to trial; DGLVP: 10 μg desglycinamide lysine vasopressin/rat s.c. 1 hr prior to trial. See further the legend to Fig. 1.

Furthermore, due to the assumption that amnesia is based on a disruption of memory consolidation, in most of these studies the drug was only administered shortly before or after application of the amnesic agent. Recent theories, however, hold that amnesia is based on a disturbance of memory retrieval [12,18]. Accordingly, the possibility exists that the administration of a drug shortly before the retrieval test may lead to a reduction of amnesia.

In the present studies these objections were partially met by examining the effect of administration of a drug prior to the acquisition trial as well as the test trial on the CO_2-induced amnesia for a step-through passive avoidance response. Elsewhere, we report similar studies, in which we used a different amnesic agent and a different behavioural task [15].

In Experiment 1 it was found that the peptide $ACTH_{4-10}$ can alleviate the amnesia when administered 1 hr prior to the test trial. Administration of the peptide 1 hr to the acquisition trial was ineffective. It is improbable that the anti-amnesic effect of $ACTH_{4-10}$ is due to an influence on locomotor capacities of the experimental animals as $ACTH_{4-10}$ did not affect the step-through latencies of non-shocked animals. $ACTH_{4-10}$ did not affect the passive avoidance behaviour of FS-No CO_2 rats. However, in the present paradigm it was not possible to measure an increased tendency to avoid as most of the placebo FS rats made already a complete avoidance response.

The results of Experiment 1 suggest that $ACTH_{4-10}$ promotes the retrieval of memory items which are affected by the amnesic treatment. This effect can be explained in three ways: (1) $ACTH_{4-10}$ strengthens the passive avoidance tendency. Such an effect may be inferred from the work of Thompson and de Wied [19]. However, Rigter and van Riezen [15] demonstrated that $ACTH_{4-10}$ is also able to reverse the CO_2-induced amnesia for a thirst-motivated response. This result indicates that an increased tendency to avoid is not sufficient to explain the reduction of amnesia caused by $ACTH_{4-10}$. (2) $ACTH_{4-10}$ promotes the retrieval of weak memory items. This explanation does not limit itself to memory items concerned with passive avoidance. It is possible that some or part of the relevant memory item(s) survive the amnesic treatment and that $ACTH_{4-10}$ facilitates the retrieval of these item(s) at the test trial. In using this explanation, one can leave undecided whether amnesia is based on a disturbance of memory consolidation or on a disturbance of memory retrieval. This explanation is in keeping with previous findings: an improved retrieval may be responsible for the facilitation of acquisition and the delay of extinction of avoidance behaviour following treatment with $ACTH_{4-10}$ [8,22]. (3) $ACTH_{4-10}$ promotes the retrieval of memory by reversing the disturbance of retrieval induced by the amnesic treatment. Explanation 3 is not necessarily incompatible with Explanation 2. To test this possibility, it is necessary to develop a behavioural test which is able to discriminate between a disturbance of memory consolidation and a disturbance of memory retrieval.

The reversal of amnesia by treatment with $ACTH_{4-10}$ did not occur in all animals. It is improbable that this is due to an inadequate dose of the peptide as in another study it was found that a dose of 10 μg $ACTH_{4-10}$/rat already exerts an anti-amnesic effect and that this effect cannot be increased by augmenting the dose to 100 μg/rat [14]. Therefore, it can be assumed that 100 μg $ACTH_{4-10}$ is an adequate dose.

In contrast to $ACTH_{4-10}$, $ACTH_{11-24}$ did not influence the CO_2-induced amnesia for the passive avoidance response. This finding suggests that the anti-amnesic effect of ACTH can be traced to the same amino acid sequence (i.e., 4–10) which has been found effective in delaying the extinction of avoidance response [22].

In keeping with the results of Lande, Flexner and Flexner [9] it was found in Experiment 3 that DG-LVP caused a reduction of the CO_2-induced amnesia for the passive avoidance response when injected 1 hr prior to the acquisition trial. Moreover, it was demonstrated that DG-LVP exerts the same effect when administered 1 hr prior to the test trial. These findings indicate that DG-LVP has a qualitatively different effect to $ACTH_{4-10}$. Other investigations have led to the same conclusion [2,24].

The finding that DG-LVP has an anti-amnesic effect when administered prior to the acquisition trial suggests that this peptide is able to promote memory consolidation either by facilitating the consolidation process or by protecting memory consolidation from the adverse effects of the amnesic treatment. However, the possibility that administration of DG-LVP prior to acquisition influences later retrieval cannot be excluded. There is some evidence in favour of this hypothesis. The fact that DG-LVP has an anti-amnesic effect when injected prior to the test trial suggests that this compound promotes memory retrieval either by facilitating the retrieval process or by reversing a CO_2-induced disturbance of retrieval. The effect of pre-acquisition treatment with DG-LVP may be based on the same mechanism.

ACKNOWLEDGEMENT

We would like to thank Miss R. Elbertse for her excellent technical assistance.

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