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Hippocampal Monoamine Metabolism and the CO_2 Induced Retrograde Amnesia Gradient in Rats[1]

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RIGTER, H., G. VAN EYS AND B. E. LEONARD. *Hippocampal monoamine metabolsim and the CO_2 induced retrograde amnesia gradient in rats.* PHARMAC. BIOCHEM. BEHAV. 3(5) 781–785, 1975. – It was found that in rats a gradient of retrograde amnesia for a passive avoidance response could be established when carbon dioxide (CO_2) was used as the amnesic agent. The extent of passive avoidance increased as the period between application of a mild foot shock and CO_2 treatment was increased. The amnesia gradient was found to cover a period of at least 60 min. Changes in hippocampal serotonin metabolism parallelled the amnesia gradient. Thus, the concentration of serotonin increased as the interval between the foot shock and the CO_2 treatment increased. The changes in hippocampal noradrenaline and dopamine did not correlate with the amnesia gradient.

Amnesia gradient Passive avoidance Carbon dioxide Hippocampus Monoamines

A common feature of retrograde amnesia in rats is the existence of amnesia gradients. This implies that amnesia for an experience is maximal when acquisition of that experience is immediately followed by the application of the amnesic agent; the degree of amnesia declines when the interval between acquisition and amnesic treatment gets longer. Such amnesia gradients have been found in studies enploying diverse amnesic agents as electroconvulsive shock [11,12] and CO_2 [14,18].

The present study is part of an investigation aimed at assessing possible associations between brain monoamine metabolism and amnesia. Previously, we found that the amnesic effect of CO_2 treatment parallelled changes in brain monoamine metabolism [10]. These changes were most pronounced in the hippocampus, a region of the brain which is thought to be associated with memory consolidation processes [13,24] and behavioral inhibition [5]. Most conspiciously, acquisition of the passive avoidance response was attended with a rise in the hippocampal concentration of serotonin 24 hr later. This rise was not observed when acquisition was immediately followed by CO_2 treatment. The CO_2 treatment alone did not produce changes in the behavioral response or the amine parameters. However, these data do not provide sufficient evidence that the changes in brain amine metabolism and amnesia are correlated phenomena. Therefore, we decided to study the effect of variations in the behavioral response on the changes in hippocampal amine levels. If these neurochemical changes are directly or indirectly related to the behavioral response, the retrograde amnesia gradient may be reflected in an amine gradient. In Experiment 1, acquisition of a passive avoidance response was followed by amnesic treatment after a delay of 0–60 min; the resulting amnesia gradient was assessed at a retrieval test 24 hr after acquisition. In Experiment 2, the same paradigm was followed but instead of subjecting the animals to a retrieval test, they were killed 24 hr after acquisition and hippocampal monoamine levels were determined.

EXPERIMENT 1

Method

Eighty male rats of an inbred Wistar strain were used. They weighed 230–260 g at the beginning of the experiment. The rats were trained in a step-through passive avoidance apparatus of the type described by Ader *et al.* [1]. This consisted of a dark chamber with a grid floor. A brightly illuminated runway protruded from the front wall. During a trial, a rat was placed at the end of the runway and allowed to enter the chamber through an opening in the front wall. The latency of the animal to enter the chamber was recorded in tenths of a second.

The animals were randomly divided into 8 groups of 10

[1] Part of this investigation was presented in a paper read to the 544th Meeting of the Biochemical Society, London, November 1973.

rats each. They were trained according to the procedure described by Leonard and Rigter [10]. They received 3 pretraining trials on Day 1 of the experiment and a single acquisition trial on Day 2. During the acquisition trial a 0.5 mA foot shock (FS) was given for 3 sec to all groups of rats. One group was given CO_2 treatment immediately after it had received the foot shock. In 6 other groups, the CO_2 treatment was delayed for 5, 10, 15, 20, 30 and 60 min after the foot shock. The method of administration of CO_2 was described previously [10,15]. The rats were treated with 100 percent CO_2 for 30–35 sec. The remaining group (FS-NoCo$_2$ group) received foot shock followed immediately by sham amnesic treatment, i.e., they were placed in an air-filled box for 35 sec.

A single retrieval test was given 24 hr after acquisition. When a rat failed to enter the chamber within 300.0 sec, it was removed from the runway and a latency score of 300.0 sec was assigned. The results were analyzed by means of the Yates test [23]. The test scores were divided into 3 classes: (1) latencies of 0-10.0 sec (no avoidance); (2) latencies of 10.1–299.9 sec (incomplete avoidance); (3) latencies of 300.0 sec (complete avoidance).

Results

Figure 1 shows that the rats in the FS-NoCO$_2$ group displayed a complete passive avoidance response. Those rats which were subjected to the CO_2 treatment immediately following foot shock, with one exception, failed to show passive avoidance behavior. It can thus be concluded that the CO_2 treatment induced amnesia. The degree of amnesia decreased as the interval between the foot shock and the CO_2 treatment increased (Fig. 1 and Table 1). Nevertheless, comparisons with the scores of the FS-NoCO$_2$ group indicated that some amnesia was present in all the CO_2 treated groups (Table 2). These results suggest that the amnesia gradient covers a period of at least 60 min.

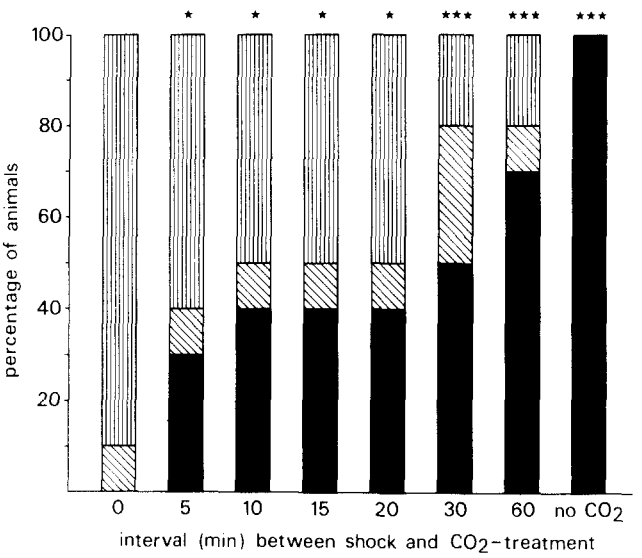

FIG. 1. Gradient of CO_2 induced retrograde amnesia. Class 1 (Vertical bars): no passive avoidance; Class 2 (Diagonal bars): incomplete passive avoidance; Class 3 (Solid bars): complete passive avoidance. Asterisks mark significant differences from the group which treated with CO_2 immediately after the foot shock: $*p<0.05$; $***p<0.001$.

EXPERIMENT 2

Method

Four groups of 20 male Wistar rats (230–250 g) were used. The experiment was run in 5 randomized blocks. Each block contained 16 rats, 4 of each group. The animals were trained as described in Experiment 1. One group of rats received neither the foot shock nor the amnesic treatment at the time of the acquisition trial (NoFS-NoCO$_2$ group). Three other groups were subjected to the CO_2 treatment 0, 15 or 60 min after they had been given the foot shock (FS-CO$_2$ groups). The remaining group received the foot shock immediately followed by sham amnesic treatment (FS-NoCO$_2$ group). The rats were not subjected to a test trial but instead were killed by decapitation 24 hr after the acquisition trial. The brains were placed on ice and the hippocampi were dissected within 2 min and frozen on solid carbon dioxide. The hippocampal samples contained the area dentata and the subiculum in addition to the hippocampus proper dorsal to the rhinal sulcus. The hippocampi from 4 rats of the same group were pooled for the biochemical assays. The tissue was homogenized in 7 ml 0.01 N HCl to which 0.7 ml 10 percent (w/v) solium edate had been added. Following centrifugation (800Xg; 20 min), aliquots of the clear supernatant were removed for the determination of noradrenaline and dopamine [3,4], tyrosine [22], tryptophan [9] and gamma-aminobutyric acid (GABA) [20]. The pellet and the remainder of the supernatant fraction were extracted with butanol for the determination of serotonin [17] and 5-hydroxyindoleacetic acid (5-HIAA) by the method of Giacolone and Valzelli [8] as modified by Tonge and Leonard [19].

Results

From the results presented in Table 3, it is apparent that as the interval between the application of the foot shock and the CO_2 treatment increases so the concentration of hippocampal serotonin also increases. In the FS-CO$_2$ group, which was subjected to the amnesic treatment immediately after the foot shock, the concentration of serotonin did not differ significantly from the control (NoFS-NoCO$_2$) group. However, the concen tration of serotonin was significantly raised both in the FS-CO$_2$ group in which the application of the amnesic treatment was delayed for 60 min after the foot shock, and in the FS-NoCO$_2$ group. The increase in the hippocampal concentration of tryptophan and 5-HIAA in these two groups did not reach statistical significance.

A biochemical gradient could not be established for tyrosine, dopamine and noradrenaline (Table 3). A slight rise occurred in the concentration of tyrosine in the FS-CO$_2$ (60 min) group and in the FS-NoCO$_2$ group but this did not reach statistical significance. Compared to the NoFS-NoCO$_2$ group, the concentration of noradrenaline was lower in the FS-CO$_2$ (0 min) and FS-CO$_2$ (15 min) groups but higher in the FS-CO$_2$ (60 min) and FS-NoCO$_2$ groups. From the results presented in Table 3 it appears that as the interval between foot shock and CO_2 treatment is prolonged, so the concentration of GABA increa-

TABLE 1

DECREASE OF AMNESIA AS A FUNCTION OF THE INTERVAL BETWEEN FOOT SHOCK AND CO_2 TREATMENT

Foot Shock–CO_2 Interval (min)	Statistical Value* (z)	Level of Significance
5	1.82	$p<0.05$
10	2.22	$p<0.05$
15	2.22	$p<0.05$
20	2.22	$p<0.05$
30	3.18	$p<0.001$
60	3.37	$p<0.001$

*The statistical values are based on comparisons with the group of rats which was treated with CO_2 immediately after the foot shock (one-tailed Yates test).

TABLE 2

PRESENCE OF AMNESIA IN RELATION TO THE DURATION OF THE INTERVAL BETWEEN FOOT SHOCK AND CO_2 TREATMENT

Foot Shock–CO_2 Interval (min)	Statistical Value* (z)	Level of Significance
0	4.37	$p<0.0001$
5	3.19	$p<0.0001$
10	2.84	$p<0.01$
15	2.84	$p<0.01$
20	2.84	$p<0.01$
30	2.39	$p<0.01$
60	1.80	$p<0.05$

*Statistical values are based on comparisons with the group of rats which was not treated with CO_2 (FS-NoCO_2). The values were calculated by means of the one-tailed Yates test.

ses. However, this increase way only significant in the FS-CO_2 (60 min) group.

DISCUSSION

The present results corroborate our previous finding that foot shock in the passive avoidance task induced an increase in the hippocampal concentration of serotonin whereas the rise in the concentration of this amine was not observed when the foot shock was immediately followed by CO_2 treatment [10]. Furthermore, the present investigation shows that the gradient for the CO_2 induced retrograde amnesia was parallelled by a gradient in the hippocampal concentration of serotonin. The effectiveness of the CO_2 treatment in inducing amnesia was decreased as the interval between administration of the foot shock and the CO_2 treatment was increased; at the same time the concentration of serotonin in the hippocampus increased. In his studies on the effect of electroconvulsive shock-induced amnesia on brain monoamine metabolism, Essman [7] found that in mice amnesia for a passive avoidance response correlated with a rise in brain

TABLE 3

CHANGES IN HIPPOCAMPAL MONOAMINE CONCENTRATIONS FOLLOWING TREATMENT WITH FOOT SHOCK AND CO_2

	Groups				
	NoFS-NoCO$_2$	FS-CO$_2$ (0 min)	FS-CO$_2$ (15 min)	FS-CO$_2$ (60 min)	FS-NoCO$_2$
Tryptophan	6.23	6.50 (−19, +34) +4%	5.18 (−36, +6) +18%	7.25 (−9, +49) +16%	7.11 (−11, +47) +14%
Serotonin	2.59	2.52 (−34, +12) −8%	3.27 (−3, +65) +26%	3.64 (+10, +80) +41%*	3.54 (+7, +75) +37%*
5-HIAA	0.31	0.26 (−27, +3) −14%	0.33 (−8, +29) +9%	0.34 (−6, +29) +10%	0.39 (−7, +48) +26%
Tyrosine	1.53	1.48 (−23, +21) −3%	1.46 (−24, +19) −5%	1.72 (−9, +41) +13%	1.82 (−4, +49) +20%
Dopamine	0.63	0.58 (−29, +19) −8%	0.63 (−22, +30) 0%	0.55 (−33, +12) −14%	0.71 (−13, +45) +12%
Noradrenaline	0.086	0.064 (−49, −5) −27%*	0.080 (−34, +24) −10%	0.114 (−4, +80) +31%	0.096 (−18, +53) +12%
GABA	144	169 (−17, +56) +14%	183 (−7, +74) +27%	213 (+8, +103) +48%*	196 (−1, +87) +36%

FS = foot shock; NoFS = no foot shock; CO_2 = CO_2-treatment given 0, 15 or 60 min after foot shock; NoCO$_2$ = no CO_2-treatment
*All statistical comparisons made between the treatment groups and the untreated group (NoFS-NoCO$_2$), $p<0.05$
The 95 percent confidence limits are shown in parentheses. All values are given in μg/g wet weight of hippocampus.

serotonin; in contrast our results indicate the absence of amnesia is correlated with a rise in the hippocampal concentration of serotonin. Differences in the species and experimental design used may be responsible for this discrepancy.

The changes in the hippocampal concentration of GABA were qualitatively similar to those of serotonin. Thus the concentration of GABA rose as the interval between the foot shock and the CO_2 treatment increased but it did not reach statistical significance in all cases.

There does not appear to be a clear correlation between the behavioral response and hippocampal catecholamine concentrations. However, the results for changes in the concentration of noradrenaline are at variance with those reported in a previous study [10] where we found a slight but significant increase in the concentration of this amine in the amnesic group. The reason for this difference is not apparent at present. It is possible that noradrenaline may be involved in amnesia. From his studies on neurochemical changes which occur in relation to electroconvulsive shock-induced amnesia in mice, Essman [6,7] concluded that a rise in brain noradrenaline may be co-incidental with amnesia. However, this change in noradrenaline may be attributed to an increase in the permeability of the blood-brain barrier [16].

From the finding that the CO_2 induced retrograde amnesia gradient is parallelled by a hippocampal serotonin gradient it can not be concluded that indoleamines play an

essential role in the formation of memory for a passive avoidance response, as suggested by Allen *et al.* [2], or in amnesia. The amnesia gradient and the hippocampal serotonin gradient may be unrelated phenomena. All measurements were made 24 hr after acquisition. The relation between the behavioral response and hippocampal serotonin metabolism may not hold for other acquisition-test intervals. Elsewhere [21], we present evidence that the gradual development of amnesia over the first 4 hr following amnesic treatment is parallelled by a gradual change in hippocampal serotonin levels. However, when the acquisition-test interval was extended beyond 24 hr, the relationship did not hold any longer; hippocampal amine levels returned to normal whereas amnesia remained present. Taken together, these result suggest that at least during the first 24 hr following CO_2 treatment, amnesia is directly or indirectly correlated with changes in hippocampal serotonin metabolism.

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