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Parallel Changes in Behaviour and Hippocampal Monoamine Metabolism in Rats after Administration of ACTH-Analogues

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RAMAEKERS, F., H. RIGTER AND B. E. LEONARD. *Parallel changes in behaviour and hippocampal monoamine metabolism in rats after administration of ACTH-analogues.* PHARMAC. BIOCHEM. BEHAV. 8(5) 547–551, 1978. – Application of a footshock during the acquisition trial of a one-trial passive avoidance test is associated with a rise in the concentration of serotonin in the hippocampi of rats 24 hr after termination of the acquisition trial. Rats subjected to amnesic treatment with carbon dioxide (CO_2) immediately after footshock do not show this rise in the hippocampal concentration of serotonin. The ACTH-analogues, ACTH 4–10 and ACTH 4–10 (7D–Phe), alleviate CO_2-induced amnesia for the passive avoidance response when administered 1 hr before a retrieval test 24 hr after acquisition. These peptides do not have anti-amnesic activity when given before acquisition. Another ACTH-analogue, ACTH 11–24, does not affect amnesia, given before either the acquisition or the retrieval test. The anti-amnesic effect of ACTH 4–10 and ACTH 4–10 (7D–Phe), was correlated with a rise in the hippocampal serotonin concentration similar to that observed in non-amnesic animals. Pre-acquisition treatment with ACTH 4–10 or administration of ACTH 11–24 did not affect hippocampal serotonin concentrations. Changes in the hippocampal concentrations of noradrenaline, dopamine, tryptophan and tyrosine were not related to the behavioural activity of any of the peptides. It is suggested that alterations in hippocampal serotonin metabolism 24 hr after acquisition of a passive avoidance response are associated with the retrievability of the passive avoidance response.

Passive avoidance Footshock Hippocampus ACTH analogues CO_2 induced amnesia
Peptides Serotonin Noradrenalin Dopamine Tryptophan Tyrosine

APPLICATION of a footshock (FS) to rats during the acquisition trial of a one-trial passive avoidance task results in passive avoidance behaviour as measured at a retrieval test 24 hr later [8]. We have previously suggested that in rats a correlation may exist between changes in hippocampal serotonin metabolism and the retrievability of this one-trial passive avoidance response [4]. Support for this view stems from studies demonstrating that experimentally induced changes in the tendency to show avoidance behaviour are generally paralleled by changes in the concentration of serotonin in the hippocampus. Acquisition of the avoidance response correlates with a rise in the concentration of serotonin in the hippocampus at least up to 24 hr after conclusion of the acquisition trial [4, 8, 12]. Retrograde amnesia for the avoidance response can be produced by treating the rats with carbon dioxide (CO_2). In amnesic rats hippocampal serotonin concentrations do not differ from control values [4, 8, 12]. The degree of amnesia declines when the time interval between application of FS and treatment with CO_2 is increased. This so-called amnesia gradient is paralleled by changes in the concentration of hippocampal serotonin: the concentration increases as the time interval between FS and CO_2 is extended [12]. In addition, we have shown that the CO_2-induced amnesia for the passive avoidance response develops gradually over the first 4 hr after amnesic treatment and that this gradual onset of amnesia is paralleled by a gradual decline of the enhanced concentration of hippocampal serotonin [4].

These findings suggest that at least up to 24 hr after acquisition a high concentration of hippocampal serotonin correlates with a strong tendency to display passive avoidance behaviour, whereas hippocampal serotonin concentrations are normal in animals in which this tendency to show avoidance behaviour is weak or absent. This is supported by the results of experiments [9] in which we

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used peptides known to influence retrograde amnesia. The vasopressin analogue desglycinamide lysine vasopressin attenuates the CO_2-induced amnesia for the passive avoidance response when administered either before the acquisition trial or before the retrieval test. We have found that this attenuation of amnesia is associated with a rise in hippocampal serotonin 24 hr after acquisition similar to that observed in rats which received FS but no CO_2-treatment [9].

The present paper provides evidence that the anti-amnesic effect of another group of peptides, i.e. ACTH-analogues, is also reflected in parallel changes in hippocampal serotonin metabolism. The amino acid sequence ACTH 4–10 lacks the corticotrophic activity of ACTH but possesses the same behavioural activity as the parent hormone [6,19]. One prominent behavioural effect of ACTH 4–10 is the attenuation of CO_2-induced amnesia for passive avoidance response. In contrast to desglycinamide lysine vasopressin, however, ACTH 4–10 alleviates amnesia only when given prior to the retrieval test but not when given before acquisition [14]. The same is true for another analogue, i.e. ACTH 4–10 (7D–Phe) [10]. It has been suggested that the amino acid sequence 4–10 contains the minimal requirements for the expression of behavioural activity [19]. This assumption receives support from the finding that ACTH 11–24 does not affect CO_2-induced amnesia [14].

As an extension of our previous investigations we assessed the following hypothesis: If alterations in hippocampal serotonin metabolism are indeed correlated with the retrievability of the passive avoidance response, administration of ACTH 4–10 or ACTH 4–10 (7D–Phe) prior to killing the rats 24 hr after acquisition and amnesic treatment (i.e., at the time the retrieval test would have been given) should restore the rise in hippocampal serotonin just as it restores passive avoidance behaviour in amnesic animals. However, treatment with these ACTH-analogues before acquisition should not affect the hippocampal concentration of serotonin. In addition, as ACTH 11–24 is virtually devoid of anti-amnesic activity it should not influence hippocampal serotonin concentrations when administered before decapitation of the animals.

METHOD

Behaviour

In each experiment 144 male Wistar rats, weighing 210–240 g, were used. The animals were obtained from the TNO breeding station, Zeist, The Netherlands. They were housed eight per cage with ad lib access to food and water.

The rats were trained in the step-through passive avoidance apparatus described by Ader *et al.* [1]. This consisted of a 40 × 40 × 40 cm dark chamber with a grid floor. An elevated runway, illuminated by a 40 W lamp, 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 a 6 × 6 cm opening in the front wall. The time taken by an animal to enter the chamber was recorded and defined as the step-through latency.

The experiments were run in eight randomized blocks. Each block contained 18 rats. The rats were trained according to the procedure described by Leonard and Rigter [8]. The animals were given three pretraining trials on Day 1 of the experiment and a single acquisition trial on Day 2. During the acquisition trial a 0.5 mA scrambled foot shock (FS) was administered through the grid floor of the chamber to four groups of rats. The shock was produced by a 500 V AC source through a variable resistance. The remaining four groups did not receive foot shock (No FS groups).

Two FS groups and two NoFS groups were anaesthesized with CO_2 immediately upon conclusion of the acquisition trial (FS-CO_2 and NoFS-CO_2 groups, respectively). The CO_2-treatment consisted of placing a rat in a box with 100% CO_2 for 30–35 sec until respiratory arrest occurred. The animal was then removed from the box, revived by artificial respiration and returned to its home cage. The rats from the remaining two FS groups and two NoFS groups were given sham amnesic treatment by placing them in an air-filled box for 35 sec (FS-NoCO_2 and NoFS-NoCO_2 groups). Previously we have shown that FS-NoCO_2 rats subjected to the same footshock displayed passive avoidance behaviour at a retrieval test 24 hr after acquisition; rats subjected to foot shock and amnesic treatment (FS-CO_2) entered the dark chamber during the retrieval test as quickly as control (NoFS-NoCO_2 and NoFS-CO_2) animals and therefore showed amnesia for the passive avoidance response.

In the present biochemical studies, instead of subjecting the rats to a retrieval trial they were decapitated 24 hr following the acquisition trial. One hr prior to acquisition or 1 hr prior to killing the animals were injected SC with 1 ml physiological saline or 100 μg/rat ACTH 4–10, ACTH 4–10 (7D–Phe) or ACTH 11–24 according to the design given in Table 1. The peptides were dissolved in a hydrochloric acid solution of pH 3.5. This solution was diluted with saline to a concentration of 100 μg/ml and neutralized with sodium bicarbonate prior to injection.

Biochemistry

After killing the rats, the heads were placed on ice. 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 dorsal to the rhinal sulcus. Hippocampi from three rats of the same treatment group were pooled for the biochemical assays.

The tissue was homogenized in 8 ml 0.01 N HCl to which 0.5 ml 10% (w/v) ethylene diamine tetracetic acid (as the sodium salt) had been added. After centrifugation (800 × g for 20 min at 4° C), aliquots of the clear supernatant were removed for the fluorimetric determination of noradrenaline and dopamine [2,3], tyrosine [18] and tryptophan [16]. The pellet and the remainder of the supernatant fraction were extracted with n-butanol for the determination of serotonin [15]. All fluorescence measurements were made using a Hitachi-Perkin spectrophotofluorimeter Model 2A. A double-blind procedure was used for the estimation of the biochemical parameters. For the statistical analysis of the results, a randomized block analysis of variance was employed. The level of significance used was $p<0.05$.

RESULTS

Table 2 shows that in all experiments saline-treated FS–NoCO_2 groups had increased hippocampal concentrations of serotonin compared with the corresponding NoFS-NoCO_2 control groups. This rise was not observed in the saline-treated FS-CO_2 or NoFS-CO_2 groups. Adminis-

TABLE 1

DESIGN OF THE EXPERIMENTS

Group	N	Foot Shock	CO_2	Treatment 1 hr Prior to Acquisition or Killing
NoFS-NoCO$_2$	18	–	–	saline
NoFS-NoCO$_2$	18	–	–	peptide
NoFS-CO$_2$	18	–	+	saline
NoFS-CO$_2$	18	–	+	peptide
FS-CO$_2$	18	+	+	saline
FS-CO$_2$	18	+	+	peptide
FS-NoCO$_2$	18	+	–	saline
FS-NoCO$_2$	18	+	–	peptide

The peptides used were ACTH 4–10; ACTH 4–10(7D-Phe); and ACTH 11–24. ACTH 4–10 was administered in separate experiments either 1 hr before acquisition or 1 hr before killing. ACTH 4–10(7D-Phe) and ACTH 11–24 were injected 1 hr before killing. The dose was 100 μg/rat SC in all instances.

TABLE 2

EFFECTS OF ACTH-ANALOGUES GIVEN BEFORE ACQUISITION OR KILLING ON THE CONCENTRATIONS OF SEROTONIN IN HIPPOCAMPI OF GROUPS OF RATS SUBJECTED TO FOOT SHOCK AND/OR CO_2

Peptide	Treatment Group					
	Saline NoFS-NoCO$_2$	Peptide NoFS-NoCO$_2$	Saline FS-CO$_2$	Peptide FS-CO$_2$	Saline FS-NoCO$_2$	Peptide FS-NoCO$_2$
ACTH 4–10 (before acquisition)	2.44	2.56 + 5%	2.66 + 9%	2.53 + 4%	2.94* +20% (+3, +39)	3.01* +23% (+6, +44)
ACTH 4–10 (before decapitation)	2.43	2.56 + 5%	2.54 + 5%	2.98* +22% (+7, +41)	2.88* +19% (+3, +36)	2.99* +23% (+7, +42)
ACTH 4–10 (7D-Phe) (before decapitation)	2.43	2.85 +17%	2.69 +11%	3.17* +31% (+10, +55)	3.02* +24% (+5, +47)	2.96* +22% (+3, +45)
ACTH 11–24 (before decapitation)	2.62	2.53 – 3%	2.70 + 3%	2.61 0	2.98 +14%	2.94 +12%

FS = foot shock NoFS = no foot shock CO_2 = CO_2-treatment NoCO$_2$ = sham amnesic treatment

*Significance of difference with respect to the saline-treated NoFS-NoCO$_2$ group: $p<0.05$

Each result represents the mean of 6 determinations (μg/g wet weight). The percentage change in each experimental group relative to the saline-treated NoFS-NoCO$_2$ group is also given, together with 95% confidence limits for significant differences (in parentheses).

tration of ACTH 4–10 1 hr prior to acquisition did not modulate hippocampal serotonin concentrations in any of the groups. Treatment with ACTH 4–10 or ACTH 4–10 (7D–Phe) 1 hr before decapitation did not affect serotonin concentrations in NoFS or FS-NoCO$_2$ groups but resulted in a significant elevation of the serotonin concentration in FS-CO$_2$ rats. ACTH 11–24, injected before decapitation, was without effect.

FS and/or CO$_2$-treatments did not alter noradrenaline, dopamine, tryptophan or tyrosine concentrations in the hippocampi of saline-treated rats. Administration of ACTH 4–10 before acquisition produced an increase in the hippocampal noradrenaline concentrations of the NoFS-NoCO$_2$ and NoFS-CO$_2$ groups (results not shown). ACTH 4–10 (7D–Phe)-treated groups had higher concentrations of tyrosine than the saline-treated NoFS-NoCO$_2$ control group but the control values were exceptionally low in this case.

DISCUSSION

In accordance with our previous findings [4, 8, 9, 12], rats subjected to foot shock but not to amnesic treatment showed increased hippocampal concentrations of serotonin 24 hr after acquisition. Such an increase did not occur when foot shock was followed by amnesic treatment (CO$_2$). CO$_2$-treatment alone did not affect serotonin concentrations.

On basis of these and similar results, we have argued elsewhere [4] that a correlation may exist between the observed changes in hippocampal serotonin metabolism on the one hand and the retrievability of the passive avoidance response on the other. Twenty-four hr after application of the foot shock rats show passive avoidance behaviour in our behavioural test and an elevated concentration of hippocampal serotonin in our biochemical assays; the absence of passive avoidance behaviour after amnesic treatment is associated with the absence of an increase in serotonin in the hippocampi of amnesic animals.

This line of research was further pursued in the present investigation by studying the effects of ACTH-analogues known to possess anti-amnesic activity. Rigter *et al.* [14] showed that the ACTH-fragment, ACTH 4–10, attenuates CO$_2$-induced amnesia for the one-trial step-through avoidance response studied in the present experiments. The anti-amnesic effect of ACTH 4–10 was only apparent when the peptide was administered sc within 8 hr before the retrieval test given 24 hr after acquisition [11]. Treatment with ACTH 4–10 prior to acquisition was ineffective [14]. A similar anti-amnesic effect was obtained with the same amino acid sequence in which the phenylalanine residue in position seven was replaced by its D-isomer [ACTH 4–10 (7D–Phe)] [10].

ACHT 4–10 and ACTH 4–10 (7D–Phe) yield similar or different behavioural effects, depending on the nature of the test. Thus, both peptides have anti-amnesic activity [10,14] and delay extinction of conditioned taste aversion [13]. However, whereas ACTH 4–10 retards extinction of shock-motivated or food-rewarded responses, ACTH 4–10 (7D–Phe) facilitates extinction of these responses [5,6]. Rigter *et al.* [14] also examined the effect of ACTH 11–24 on amnesia. This peptide has no appreciable influence on the extinction of shock-motivated responses [6]. Similarly, ACTH 11–24 does not affect CO$_2$-induced amnesia [14].

The rationale of the present experiments was that, if changes in hippocampal serotonin concentrations are indeed associated with the retrievability of the passive avoidance response, treatment with both ACTH 4–10 and ACTH 4–10 (7D–Phe) 1 hr before decapitation should result in a rise in hippocampal serotonin, similar to the one observed in non-amnesic FS rats.

On the other hand, pre-acquisition treatment with ACTH 4–10 or pre-decapitation treatment with ACTH 11–24 should be without effect. These predictions were confirmed. Administration of ACTH 4–10 or ACTH 4–10 (7D–Phe) prior to decapitation produced an increase in hippocampal serotonin in FS-CO$_2$ groups. This increase was comparable to the one observed in FS-NoCO$_2$ groups. The peptides did not alter hippocampal serotonin concentrations in any of the other groups. ACTH 11–24 was inactive.

The ACTH-analogues did not produce consistent changes in any of the other neurochemical parameters. A possible exception may be the rise in hippocampal noradrenaline concentrations which was found in NoFS groups after pre-acquisition treatment with ACTH 4–10. This effect cannot be readily explained by the increase in noradrenaline turnover which has been found in rat brain after treatment with ACTH 4–10 [7,17].

The present results provide further support for the view that 24 hr after FS a correlation exists between the retrievability of the avoidance response in the one-trial passive avoidance test and hippocampal serotonin metabolism. This conclusion cannot be taken to mean that serotonin plays a causal or an exclusive role in the mediation of passive avoidance behaviour. It is noteworthy in this respect that the correlation between changes in hippocampal serotonin and passive avoidance behaviour no longer holds when the time interval between acquisition and the retrieval test is extended beyond 24 hr. Hippocampal serotonin concentrations then return to normal whereas avoidance behaviour and amnesia remain [4]. This suggests that the correlation between alterations in hippocampal serotonin metabolism and the retrievability of the passive avoidance response only exists within certain time limits.

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