ACTH, α-MSH and β-LPH: pituitary hormones with similar activity in an amnesia test in rats

H. RIGTER*, S. SHUSTER**, A. J. THODY**, *Department of Pharmacology, Scientific Development Group, Organon International b.v., Oss, The Netherlands and **Department of Dermatology, The Royal Victoria Infirmary, The University of Newcastle upon Tyne NE1 4LP, U.K.*

In addition to its classical endocrine effect, adrenocorticotrophic hormone (ACTH) also influences behaviour through direct effects on the brain. Thus its administration to rats during the extinction of shock-motivated responses (Greven & de Wied, 1973) or food-motivated responses (Garrud, Gray & de Wied, 1974) delayed the extinction. Extirpation of the adrenal gland did not abolish the behavioural effect of ACTH (Miller & Ogawa, 1962). Moreover, fragments, e.g. $ACTH_{4-10}$, and analogues lacking the endocrine activity of ACTH still appeared to have behavioural actions similar to the parent hormone (Greven & de Wied, 1973; de Wied, Witter & Greven, 1975). Besides, corticosteroids in general have been found to exert behavioural effects opposite to those of ACTH-like peptides (Bohus, 1970; van Wimersma Greidanus, 1970; Garrud & others, 1974).

Using the pole jump test, de Wied and associates sought the smallest amino acid sequence of ACTH that possessed essentially the same behavioural activity as the parent hormone and found $ACTH_{4-7}$ to meet the essential requirements for the inhibitory effect on extinction (Greven & de Wied, 1973; de Wied & others, 1975). This amino acid sequence is shared by several pituitary hormones, including melanocyte-stimulating hormone (MSH). Both α-MSH and porcine β-MSH affect extinction of shock-motivated (Greven & de Wied, 1973) and food-motivated responses (Kastin, Sandman & others, 1975) in the same way as ACTH.

In addition to extinction of shock- or food-motivated responses, several other paradigms have been used to assess the behavioural effects of ACTH-like peptides. We have studied the effect of $ACTH_{4-10}$ on carbon dioxide (CO_2)-induced amnesia for a one-trial passive avoidance response. When during the so-called acquisition trial of the passive avoidance test a rat is punished by an electric footshock for performing a preferred response, i.e. entering a dark chamber, it usually avoids making that response again on a subsequent trial (retrieval trial). CO_2 was able to induce amnesia for this passive avoidance behaviour, when it was administered immediately upon the acquisition trial. The CO_2-induced amnesia could be attenuated by subcutaneous treatment with $ACTH_{4-10}$ 1 h before the retrieval trial. Given 1 h before the acquisition trial the peptide was ineffective (Rigter, van Riezen & de Wied, 1974). Since α-MSH contains the amino acid sequence $ACTH_{4-10}$, it may be assumed that it also possesses anti-amnesic activity. The present study was undertaken to test this hypothesis. In addition, we examined the effect of β-lipotrophic hormone (β-LPH), a pituitary hormone that also contains the sequence $ACTH_{4-10}$ (= β-LPH_{47-58}).

In each experiment 7 groups of 10 male Wistar rats, 170–210 g, were trained in the one-trial passive avoidance apparatus described by Ader, Weijnen & Moleman (1972). This consisted of a 40 × 40 × 40 cm Lucite chamber with black walls and a grid floor. A 6 cm wide, 25 cm long elevated runway protruded from the front wall of the chamber. The runway was illuminated by a 40 W lamp while the chamber was dark. When placed on the runway, a rat could enter the dark chamber through a 6 × 6 cm opening. A scrambled footshock (0·5 mA for 3 s) could be delivered through the grid floor of the chamber.

On both day 1 and day 2 of each experiment the animals received two pretraining trials during which the rat was placed at the end of the runway and the time it took to enter the chamber was recorded and defined as the step-through latency. On day 3 a single acquisition trial was given. This was similar to a pretraining trial except that the rats received a footshock (FS) 10 s after entering the chamber. Immediately after the application of the FS the animals were subjected to amnesic treatment with CO_2 (FS-CO_2 groups) or to 'sham' amnesic treatment (FS-NoCO_2 groups) according to the procedure described by Rigter & others (1974). Amnesic treatment consisted of placing the rats in a box with 100% CO_2 until respiratory arrest occurred. They were then revived by artificial respiration. Sham-treated rats were placed in an identical but air-filled box. Since our previous work has shown that CO_2 itself does not affect later performance (Rigter & others, 1974), control groups receiving CO_2 but no FS were not included in the present experiments. On day 4 (24 h after acquisition) a single retrieval trial was given and the step-through latency recorded. When a rat did not enter the chamber within 180 s, it was taken from the runway and a score of 180 s was assigned.

$ACTH_{4-10}$, α-MSH, ovine β-LPH (10 μg per rat) or saline were injected subcutaneously 1 h before the acquisition trial and/or the retrieval trial according to the schedule given in Table 1. $ACTH_{4-10}$ in a hydrochloric acid solution of pH 3·5 was diluted with saline to a concentration of 10 μg ml^{-1} and the solution neutralized to pH 7 with sodium bicarbonate before injection. α-MSH and β-LPH were dissolved in saline. β-LPH was a generous gift of Dr C. H. Li, Hormone Research Laboratory, University of California, San Francisco.

* Correspondence.

Table 1. *The influence of $ACTH_{4-10}$, α-MSH and β-LPH (10 μg per rat) on CO_2-induced amnesia for a one-trial passive avoidance response.* (A = no avoidance. B incomplete avoidance. C compete avoidance.)

Group (n = 10)	Treatment 1 h before: acquisition	retrieval	% of rats showing: A	B	C
FS-CO_2	saline	saline	80	20	0
FS-CO_2	$ACTH_{4-10}$	saline	90	10	0
FS-CO_2**	saline	$ACTH_{4-10}$	30	60	10
FS-CO_2**	$ACTH_{4-10}$	$ACTH_{4-10}$	30	50	20
FS-NoCO_2***	saline	saline	0	30	70
FS-NoCO_2***	$ACTH_{4-10}$	saline	10	10	80
FS-NoCO_2***	saline	$ACTH_{4-10}$	0	30	70
FS-CO_2	saline	saline	100	0	0
FS-CO_2	α-MSH	saline	90	0	10
FS-CO_2*	saline	α-MSH	60	40	0
FS-CO_2**	α-MSH	α-MSH	33·3	55·5	11·1
FS-NoCO_2***	saline	saline	30	20	50
FS-NoCO_2***	α MSH	saline	20	0	80
FS NoCO_2***	saline	α-MSH	0	20	80
FS-CO_2	saline	saline	70	30	0
FS-CO_2	β-LPH	saline	60	40	0
FS-CO_2†*	saline	β-LPH	33·3	44·4	22·2
FS-CO_2**	β-LPH	β-LPH	10	50	40
FS-NoCO_2***	saline	saline	0	20	80
FS-NoCO_2***	β-LPH	saline	0	20	80
FS-NoCO_2***	saline	β-LPH	0	40	60

$ACTH_{4-10}$, α-MSH and β-LPH (10μg per rat) were studied in separate experiments.
†1 animal lost due to CO_2, *: difference to corresponding saline-saline FS-CO_2 group: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

The test scores were divided into three classes: (1) latencies of 0–10 s; (2) latencies of 11–179 s; (3) latencies of 180 s. Rats entering the chamber within 10 s were considered as not showing passive avoidance; previous experiments had shown that 10 s represented the maximum latency for rats which had not received a footshock at the time of the acquisition trial.

Rats entering the chamber between 11–179 s displayed incomplete passive avoidance while those that failed to enter within 180 s were considered to show a complete passive avoidance response. In the analysis of the results, the three classes received a weighting of 0, 1 and 2, respectively. The retrieval scores were analysed with the one-tailed Yates test (Yates, 1948).

The results are shown in Table 1. In accordance with our previous finding (Rigter & others, 1974), $ACTH_{4-10}$ attenuated CO_2-induced amnesia when administered before the retrieval trial but not when given before the acquisition trial. The group of rats treated with the peptide before both acquisition and retrieval did not differ statistically from the group receiving the peptide only before the retrieval trial. $ACTH_{4-10}$ did not change passive avoidance behaviour in the FS-NoCO_2 groups.

α-MSH and β-LPH exerted an anti-amnesic effect which was essentially similar to that of $ACTH_{4-10}$; both hormones reduced amnesia but only when given before the retrieval trial. Although treatment before both acquisition and retrieval tended to increase the anti-amnesic effect, this was not statistically significant. α-MSH and β-LPH did not affect the retrieval scores of FS-NoCO_2 rats.

These results indicate that pituitary hormones sharing the amino acid sequence $ACTH_{4-7}$ (and $ACTH_{4-10}$) have similar behavioural actions in our amnesia test. This is in agreement with the results of Greven & de Wied (1973) who demonstrated a similar inhibitory effect of ACTH, α-MSH and β-MSH on extinction of a shock-motivated response. In the present experiments $ACTH_{4-10}$, α-MSH and β-LPH were administered in a dose of 10 μg per rat. Dose-response studies are needed to assess possible differences in potency.

β-LPH has received considerable attention lately as oligopeptides derived from the C-terminal part of this hormone (amino acid sequence 61–91) appeared to have strong morphinomimetic actions (Hughes, Smith & others, 1975; Cox, Goldstein & Li, 1976; Lazarus, Ling & Guillemin, 1976;. It has been suggested that β-LPH functions as a prohormone for peptides with morphinomimetic activity (Cox & others, 1976; Lazarus & others, 1976). The present data raise the interesting question of whether β-LPH also functions as a prohormone for ACTH-like peptides.

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