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The Inhibitory Effect of ACTH 1-10 on Extinction of a Conditioned Avoidance Response: Its Independence of Thyroid Function

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DE WIED, D. AND G. PIRIE. *The inhibitory effect of ACTH 1–10 on extinction of a conditioned avoidance response: its independence of thyroid function.* PHYSIOL. BEHAV. 3(2) 355–358, 1968.—The effect of the ACTH analogue ACTH 1–10 was studied on the rate of extinction of a pole jumping avoidance response in thyroidectomized rats with or without replacement therapy with l-thyroxin. ACTH 1–10 appeared to delay the rate of extinction of the CAR in thyroidectomized rats in a similar way as in sham-operated animals. ACTH 1–10 was also active in thyroidectomized rats treated with thyroxin. The thyroxin treatment itself also caused a significant delay in the rate of extinction of the CAR. These results indicate that the inhibitory effect of ACTH analogues on the rate of extinction of the CAR is not mediated by the thyroid gland.

Thyroidectomy ACTH analogue (ACTH 1–10) Extinction Pole jumping avoidance response

IN RECENT years attention has been focused upon the effect of ACTH and related peptides on conditioned avoidance behavior [1, 17, 19, 22, 28, 29]. These peptides delay extinction of a conditioned avoidance response (CAR) in rats. Similar ACTH-analogues have been shown to stimulate thyroid gland activity [3, 7, 9, 27]. The thyroid seems implicated in acquisition and retention of avoidance behavior as well as in spontaneous behavior [2, 12, 13, 14, 15, 16, 18, 21, 23, 24]. It might be possible therefore that ACTH-analogues exert their influence on avoidance behavior by mediation of the thyroid gland. For this reason, experiments were performed on the effect of an ACTH-analogue (ACTH 1–10) on the rate of extinction of an avoidance response in adult thyroidectomized rats with and without replacement therapy with thyroxin.

MATERIALS AND METHODS

Male white rats of an inbred Wistar strain weighing between 105 and 120 g were used. Thyroidectomy was performed under ether anesthesia. An incision in the skin above the trachea was made. The thyroid gland including the parathyroids was carefully dissected free from the trachea and removed. Sham-operated rats served as controls. Animals were allowed to recover from the operation for 10 days. Thereafter avoidance conditioning was begun. The efficacy of the operation was determined by macroscopic inspection of the trachea region for thyroid remnants at the end of each experiment. Data from animals with remnants were discarded. One group of thyroidectomized rats received replacement therapy with l-thyroxin. This was given in a dose of 10 μg subcutaneously every other day from the day following the operation till the last day of the experiment.

Avoidance conditioning was performed in a pole jumping situation as described earlier [29]. Rats were conditioned to jump onto a pole. The conditioned stimulus (CS) was a light emitted by a 60 W bulb on top of the cage, which was presented for 5 sec. A rat which did not jump onto the pole within 5 sec and remained on the grid floor of the cage, received an electric shock until the animal made the correct response and which served as the unconditioned stimulus (US). Ten trials were given each day for 3 days with an intertrial interval averaging 60 sec. Rats which scored 10 or more out of 30 trials were used for extinction trials. Extinction was studied for 2 days (20 trials) and in one experiment for 3 days (30 trials).

The total number of CAR's scored by each rat during either acquisition or extinction served as an index of avoidance behavior. The significance of the differences between groups were determined with the aid of Wilcoxon's two sample test [30].

The ACTH-analogue ACTH 1–10 was used. It was administered as a long-acting Zn phosphate preparation [29]. The treatment was started on the 3rd day of the acquisition period immediately following the last trial of the session. A single subcutaneous injection of 10 μg of this peptide in a suspension of 0.5 ml was given. Zn phosphate complex without peptide was used as the placebo.

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TABLE 1
EFFECT OF THYROIDECTOMY ON ACQUISITION AND EXTINCTION OF A POLE JUMPING AVOIDANCE RESPONSE AND ON BODY WEIGHT OF MALE RATS

	Number of CAR's			
	Acquisition	Extinction	Number of rats	Body weight gain (g)
Sham-operated rats	15 ± 0.9*	9 ± 1.6	14	56 ± 3.8
Thyroidectomized rats	15 ± 0.7	7 ± 1.4	13	19 + 3.4

*Mean ± Standard Error of the Mean.

RESULTS

In the first experiment, the effect of thyroidectomy was studied on avoidance acquisition and extinction as compared to sham-operation.

In total, 24 thyroidectomized and 18 sham-operated rats were used. Six thyroidectomized animals had to be discarded because of thyroid remnants. From the remaining 18 rats, 5 did not reach the conditioning criterion. In the sham-operated group 4 animals failed to reach the conditioning criterion. No significant difference in acquisition or retention of the CAR was found between thyroidectomized and sham-operated rats (Table 1). Although thyroidectomized rats tended to extinguish somewhat faster, the difference between the number of CAR's during extinction in both groups was not significant.

Body weight gain seemed normal in sham-operated rats during the 15-day period of observation. However, thyroidectomized animals had a markedly inhibited growth rate.

In the second experiment, the effect of ACTH 1–10 was studied on extinction of the CAR in thyroidectomized rats, with or without replacement therapy, and in sham-operated animals (Table 2). Avoidance learning was identical in the three groups of animals. However, in this experiment extinction of the thyroidectomized rats was significantly faster ($p < 0.01$). Treatment of thyroidectomized rats with thyroxin significantly delayed extinction of the CAR ($p < 0.01$).

The administration of ACTH 1–10 significantly delayed extinction of the CAR in sham-operated as well as in thyroidectomized rats. In thyroidectomized rats treated with thyroxin the difference between peptide treated and controls was not significant. This was due to the fact that these rats made a large number of positive avoidances during the two days of extinction which probably could not be further augmented by peptide treatment. These animals were therefore subjected to extinction for another day. Under these conditions, the inhibitory effect of ACTH 1–10 became manifest.

Body weight gain of thyroidectomized rats was markedly inhibited. Treatment with thyroxin restored growth rate of thyroidectomized rats to a considerable extent.

Table 3 summarizes intertrial response activity of sham-operated and thyroidectomized rats with and without replacement therapy during acquisition and extinction trials in the foregoing experiment. Total number of intertrial responses during the 3 acquisition or the 2 extinction sessions served as an index of intertrial response activity. Intertrial responses were not significantly different during either acquisition or extinction between sham-operated and thyroidectomized rats. Treatment with l-thyroxin significantly increased the number of intertrial responses during acquisition in one group of thyroidectomized rats treated with thyroxin. The administration of ACTH 1–10 did not affect intertrial response activity during extinction in sham-operated animals. However, the number of intertrial responses of thyroidectomized rats during extinction significantly increased during the treatment with ACTH 1–10. In fact, the peptide appeared to bring the number of intertrial responses of thyroidectomized rats back to normal.

DISCUSSION

The results show, that the ACTH-analogue (ACTH 1–10) delays extinction of a pole jumping avoidance response not only in sham-operated rats but also in thyroidectomized rats with or without replacement therapy with l-thyroxin. Accordingly, the inhibitory action on the rate of extinction of the CAR by ACTH 1–10 and other ACTH analogues which has been demonstrated before [1, 17, 29] is not mediated by an alteration in thyroid function.

Courrier and Cehović [9] were the first to draw attention to the fact that α- and β-MSH both accelerate the disappearance of 131 J from the thyroid indicating that these ACTH-analogues stimulate the release of thyroid hormones. Similar findings were reported by Cehović [7] for guinea pigs. Bowers *et al.* [3] using 131 J uptake by the thyroid in mice found increased uptake in animals treated with α- and β-MSH. Since these peptides were also effective in hypophysectomized mice, the locus of action might be in the thyroid and not in the anterior pituitary via TSH-release. However, recently Cehović [8] provided also evidence for the pituitary as the site of action of MSH-peptides in this respect.

In the present study ACTH 1–10 was used. Cehović [7] reported that this decapeptide which has a weak melanophorotrophic effect in contrast to α-MSH failed to activate the thyroid gland in guinea pigs. This author used acetyl ACTH 1–10 while we used ACTH 1–10. Recently Schally *et al.* [25] studied 7 synthetic peptides related to α-MSH and ACTH. These authors also failed to find an effect of the decapeptide although the sequence 5–10 and 7–13 were associated with TSH-like activity. It was suggested that rapid inactivation in the bloodstream and other factors may be responsible for negative effects. In the present study long-acting Zn phosphate ACTH 1–10 was used. Such a complex is for a great deal protected against enzymic destruction.

Thyroidectomy in the present experiments did not interfere with the rate of acquisition of the avoidance response. However, extinction in one experiment was facilitated. Treatment of thyroidectomized rats with thyroxin did not accelerate acquisition but it delayed extinction. Data on the effect of thyroidectomy on learning and retention of avoidance behavior are contradictory. A lack of effect of hypo- or

TABLE 2
EFFECT OF ACTH 1-10 ON EXTINCTION OF A POLE JUMPING AVOIDANCE RESPONSE IN SHAM-OPERATED AND THYROIDECTOMIZED RATS WITH AND WITHOUT REPLACEMENT THERAPY

	Number of CAR's		Number of rats	Body weight gain (g)
	Acquisition	Extinction		
Sham-operated rats				
placebo (0.5 ml)	17 ± 0.7*	10 ± 0.8	13	60 ± 10.0
ACTH 1-10 (10 μg)	18 ± 0.8	15 ± 0.9 $p < 0.02$	13	58 ± 8.8
Thyroidectomized rats				
placebo (0.5 ml)	18 ± 0.9	5 ± 1.1	10	−1 ± 9.4
ACTH 1-10 (10 μg)	19 ± 0.7	15 ± 1.5 $p < 0.01$	11	5 ± 8.0
Thyroidectomized rats with thyroxin				
placebo (0.5 ml)	19 ± 0.5	16 ± 1.2	10	41 ± 5.1
ACTH 1-10 (10 μg)	18 ± 0.6	18 ± 1.4† 17 ± 0.6 23 ± 0.5† $p < 0.02$	11	47 ± 6.0

*Mean ± Standard Error of the Mean.
†Total number of CAR's of 30 trials.

TABLE 3
INTERTRIAL RESPONSE ACTIVITY DURING ACQUISITION AND EXTINCTION OF THE CAR OF SHAM-OPERATED AND THYROIDECTOMIZED RATS WITH OR WITHOUT REPLACEMENT THERAPY

	Number of intertrial response			Number of rats
	Acquisition		Extinction	
Sham-operated rats				
placebo (0.5 ml)	5 ± 0.9*		7 ± 1.1	13
ACTH 1-10 (10 μg)	7 ± 1.3		7 ± 0.9	13
Thyroidectomized rats				
placebo (0.5 ml)	8 ± 1.7	$p < 0.01$	4.5 ± 1.3 $p < 0.05$	10
ACTH 1-10 (10 μg)	8 ± 2.1	$p = 0.05$	8 ± 1.0	11
Thyroidectomized rats with thyroxin				
placebo (0.5 ml)	13 ± 1.6		7 ± 1.5	10
ACTH 1-10 (10 μg)	10 ± 1.3		8 ± 1.2	11

*Mean ± Standard Error of the Mean.

hyperthyroidism on acquisition and retention has been reported [5, 10, 11, 20]. Thyroidectomy has been found to reduce the rate of learning as well as the rate of extinction [21, 23, 24, 26] and thyroxin has been shown to accelerate avoidance acquisition [2, 21, 23], to be deleterious to learning of a brightness discrimination [26] or to reduce retention in a maze learning situation [6]. These discrepancies may be the result of differences in the rate of thyroid deficiency, the duration of thyroid hormone deprivation, the amount of thyroxin administered and the duration of the treatment, the difference in age of the animals [12] and the respective techniques used by the various investigators.

Thyroidectomy may result in a decreased level of activity while hyperthyroidism may result in an opposite effect [4, 24]. Intertrial response activity was decreased in thyroidectomized rats and increased in one experiment in which thyroidectomized rats had received thyroxin. It might be that the treatment had made the rats slightly hyperthyroid. The fact that this increased activity was found during acquisition only cannot be explained at the present. The low intertrial response activity found in the thyroidectomized rat during extinction was brought back to normal by thyroxin administration but also by treatment with ACTH 1-10. The peptide did not affect intertrial response activity *per se* since this parameter was not affected in sham-operated rats. Intertrial interval activity might be a measure of the level of general activity. It might also be a measure of the drive state of the animal, maybe the fear drive, or the ability to discriminate. At present it is not possible to determine which of these aspects was affected by thyroxin and ACTH 1-10.

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