Although no definite hyperthermic effect was observed after injection of "exercise" plasma into the recipient animals, the result can hardly be interpreted as negative. The temperature curve of these animals was statistically significantly higher than that of the controls. The following alternative explanation can be suggested: Either during exercise, certain pyrogen-like substances appear in the blood plasma or exercise leads to disappearance from the plasma of a certain factor causing the temperature to fall in the control experiments. The second explanation, however, seems less likely because after injection of the "exercise" plasma and a 20-min delay the temperature curve fell and by the 40th minute it was at approximately the same level as in the control experiments. The results can therefore more easily be explained on the grounds that "exercise pyrogens" exist. Their chemical nature and mechanism of action require further study.

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INHIBITION OF CORTICOSTEROID PRODUCTION BY PINEAL FACTOR

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The effect of a factor isolated from the bovine pineal gland on the blood corticosteroid level and on the ability of isolated adrenal tissue to synthesize these compounds was investigated in experiments on male Wistar rats. The method of obtaining the pineal fraction is described. Its injection in a dose of 2 mg/100 g body weight lowered the blood corticosteroid level by 74, 69, 40, 64, and 63% after 4, 5, 7, 9, and 12 days respectively. The ability of the adrenal tissue to synthesize corticosteroids was depressed. The results are evidence that the pineal gland participates in the regulation of adrenal function.

KEY WORDS: adrenal glands; steroid production; pineal factor.

Much attention has recently been paid to relations between the pineal gland and other endocrine structures. In particular, research aimed at discovering the role of the pineal gland in the regulation of adrenal function is very interesting. One of the first pieces of indirect evidence that these two glands may be closely inter-

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TABLE 1. Blood 11-Hydroxycorticosteroid (11-HCS) Level after Injection of Pineal Factor (10 experiments)

Duration of treatment	11-HCS concentration, μg/100 ml			
	control (M ± m)	experiment (m ± m)	% decrease	P
4 h	21,4±0,71	5,5±0,91	—74	<0,001
5 days	21,9±0,32	6,9±1,19	—69	<0,001
7 »	21,6±0,26	12,7±1,02	—40	<0,001
9 »	19,8±0,16	7,2±0,14	—64	<0,001
12 »	21,9±0,19	5,9±0,98	—63	<0,001

TABLE 2. Corticosteroid Formation by Adrenal Tissue (10 experiments)

Duration of treatment	Activity of corticosteroid formation, μg/100 ml tissue · h			
	control (M ± m)	experiment (M ± m)	% decrease	P
4 h	64,2±0,35	41,5±0,26	—36	<0,001
12 days	69,5±0,41	40,3±0,39	—40	<0,001

connected was the discovery of a marked increase in the corticosteroid level in animals after pinealectomy [5]. It has also been shown that unpurified pineal extracts, depending on the method of their preparation, may have either an inhibitory [3, 4] or an activating [6] effect on corticosteroid biosynthesis and secretion. It is accordingly important to develop effective ways of obtaining separate fractions of pineal tissue with high activity in one or other direction, in order to continue the study of the chemical nature of the active substances and the mechanism of their action.

In this paper a new way of isolating a purified fraction from bovine pineal tissue containing a factor highly active in inhibiting adrenocortical function, is described.

EXPERIMENTAL METHODS

The original material for isolation of the factor was bovine pineal glands, 0.5 kg of which was homogenized in the cold; the homogenate was then extracted with 2.5 liters acetone, cooled to 0°C. The residue was filtered after 4 h and dried in vacuo. The dry powder was suspended in 4 liters of cold water and the pH of the mixture slowly adjusted to 12.0 with 1N NaOH. The mixture was stirred with constant cooling for 24 h, the insoluble material was removed by centrifugation for 20 min at 10,000g, and 1N HCl was added to the supernatant to bring its pH to 7.4. The precipitate formed in the course of 9 h was removed by centrifugation and the pH of the supernatant lowered to 4.5. The residue thus formed was separated after 15 h by filtration and lyophilized. The yield of the preparation was 0.3% of the original suspension of wet tissue.

The activity of the preparation was determined on male Wistar rats weighing 180-220 g. The preparation was injected intramuscularly in a dose of 2 mg/100 g body weight in 1 ml physiological saline either once or repeatedly. Control animals received injections of physiological saline. The animals were decapitated 4 h after the single injection of the preparation and their blood corticosteroid level determined. The blood corticosteroid level in the animals receiving multiple injections was determined 4 h after each injection. In both series of experiments the ability of the adrenal tissue to synthesize corticosteroids was determined. Corticosteroid-synthesizing activity of the adrenal tissue was determined as follows: A 2% homogenate of the tissue was made up in 0.25M sucrose solution at 4-5°C; 0.5 ml of the homogenate was incubated with 50 μg progesterone in 4 ml 0.002M Tris-buffer, pH 7.4, containing 5 μM $MgCl_2$, 5 μM nicotinamide, 0.317 μM NADPH, and 10 μM sodium fumarate.

The corticosteroid levels in the animals' blood and in the incubation medium after incubation were determined fluorometrically [2].

EXPERIMENTAL RESULTS AND DISCUSSION

Injection of the pineal preparation considerably reduced the blood corticosteroid level of the animals after both single and repeated injections (Table 1). Meanwhile corticosteroid formation in isolated adrenal tissue was considerably reduced concurrently (Table 2).

An inhibitory effect of pineal extracts on corticosteroid formation has been described in the literature [1]. The authors concerned state that injection of pineal extracts if followed after 4 h by a decrease of 24% in the blood corticosteroid concentration. Injection of the pineal preparation obtained by the method described above led to a decrease of 74% in the blood corticosteroid level after the same time. Stronger inhibitory activity of the preparation also was found after its repeated injection.

It can be concluded from these results that pineal factors can participate in the regulation of corticosteroid biosynthesis in the adrenal tissues. Since the effect of injection of pineal factor under the experimental conditions used was of short duration (the blood corticosteroid level was back to normal 24 h after injection of the preparation) it can tentatively be suggested that its action is realized at the hypothalamic (by inhibition of corticoliberin secretion) or at the adenohypophyseal (by inhibition of corticotrophin liberation) level. Further investigations are necessary to determine the mechanism of the physiological action of pineal factors and also to establish the chemical nature of active pineal compounds.

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