A HYPERKALIEMIC FACTOR FROM THE PINEAL GLAND

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A factor which, if injected parenterally into rats, induces hyperkaliemia has been isolated from the pineal tissue. The factor also retards the restoration of the normal plasma potassium concentration after intraperitoneal loading with potassium chloride. The substance is active in small doses. After proteolysis of the factor by pronase and trypsin, its activity is completely lost. Preparations from tissues of the cerebral cortex and liver obtained in the same way as from the pineal gland do not possess the hyperkaliemic action.

There is experimental evidence that the pineal gland is an endocrine organ which participates in the regulation of water and mineral metabolism. In particular, administration of pineal extract activates the secretion of aldosterone and other mineralocorticoids by the adrenals [1, 2]; the pineal gland contains principles which act on the level of diuresis [3]; pinealectomy leads to changes in the blood serum sodium concentration [4], a decrease in the potassium concentration in the muscles, and increased excretion of potassium with the urine [5].

In the course of an investigation of the role of the pineal gland in water and mineral metabolism the writers found and isolated a protein-peptide factor possessing hyperkaliemic action from bovine pineal tissue. This paper describes results demonstrating the physiological activity of this factor.

EXPERIMENTAL METHOD

Bovine pineal glands were used for the isolation and purification of the factor. An acetone powder was prepared from the tissues of the pineal glands and extracted three times with 0.05-M ammonium bicarbonate (pH 9.5). The alkaline extracts were discarded. The residual material was extracted twice with 0.2-M ammonium acetate (pH 4.5). These acid extracts were lyophilized, redissolved in 0.5% acetic acid, and treated by gel-filtration in a column with Sephadex G-15. By gel-filtration the acid extract was divided into five components (Fig. 1). Hyperkaliemic activity was concentrated in fraction I (shaded in Fig. 1). Further purification of fraction I was carried out by ion-exchange chromatography on a column with CM-cellulose. The conditions of chromatography and the result of division of this fraction are given in Fig. 2. The active fraction was eluted at 0.2 M in an ammonium acetate gradient.

To test the biological action of the factor, female Wistar rats weighing 150-160 g were used. The potassium concentration was determined in heparinized blood plasma by flame photometry. Blood was taken from the orbital sinus. The doses of the factor and the duration of its action on the rats varied depending on the purpose of the experiment (see below).

EXPERIMENTAL RESULTS

A hyperkaliemic effect of the factor was detected and it depended on the duration of action of the substance. The dose of the factor was 20 μg. The material was dissolved in 0.5 ml physiological saline before

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TABLE 1. Effect of Pineal Factor on Potassium Concentration in Blood Plasma

Duration of action	Potassium concentration (in meq/liter)		P
	control	experiment	
4 h	3,7±0,07	3,8±0,15	0,1
7 h	3,68±0,22	3,8±0,34	0,1
1 day	3,15±0,22	4,42±0,13	0,002
3 days	4,01±0,12	5,23±0,19	0,001
5 days	4,05±0,13	5,52±0,18	0,001
7 »	3,86±0,17	5,183±0,21	0,001
11 »	3,9±0,08	5,7±0,12	0,001

TABLE 2. Specificity of Hyperkaliemic Effect of Pineal Factor

Substance given	Dose (in μg)	Potassium concentration (in meq/liter)		P
		control	experiment	
Control (physiological saline)		3,3±0,03	—	
From pineal gland	20	3,3±0,03	5,08±0,110	0,001
From liver	5000	3,3±0,03	3,43±0,205	0,1
From cerebral cortex	4000	3,8±0,07	3,95±0,114	0,1
Melatonin	20	3,6±0,08	3,22±0,260	0,1

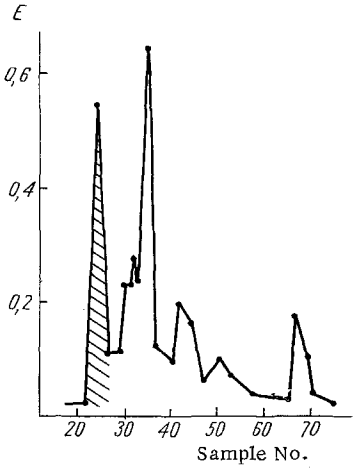

Fig. 1

Fig. 1. Fractionation of acid extract of pineal gland on column with Sephadex G-15. Column measures 2.5 × 100 cm, elution with 0.5% acetic acid, rate 90 ml/h, sample volume 5 ml.

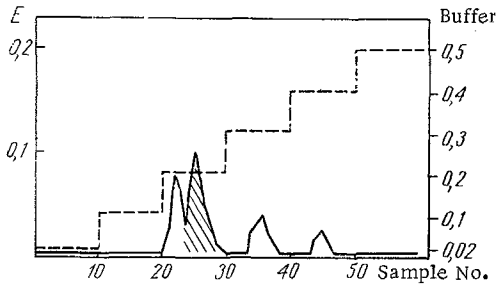

Fig. 2

Fig. 2. Chromatography of fraction I of acid extract on column with CM-cellulose. Column measures 1.2 × 15 cm. Stepwise gradient of ammonium acetate, pH 5.0, rate of elution 15 ml/h, sample volume 3.5 ml.

TABLE 3. Effect of Pineal Factor on Potassium Concentration in Blood Plasma of Adrenalectomized Rats

Group of rats	Potassium concentration in plasma (meq/liter)	P
Intact	3,85±0,10	
Control (adrenalectomy)	4,7±0,19	0,01
Adrenalectomy + KCl	5,48±0,30	0,001
Adrenalectomy + KCl + factor	6,36±0,21	0,001

administration and was given daily, alternately by subcutaneous and intramuscular injections. The results of these experiments are given in Table 1.

As Table 1 shows, an increase in the blood-plasma potassium concentration in response to injection of the factor occurs after about 24 h and it becomes stabilized at a level on the average 1.5 meq/liter higher than in the control.

The results for the hyperkaliemic action of the factor were particularly demonstrative in experiments performed in accordance with the scheme described below. Twice, at intervals of 12 h, the rats received an injection of 20 μg of the factor, followed 24 h later by the factor and 40 mg potassium chloride intraperitoneally. The dynamics of the changes in the plasma potassium concentration of the experimental and control groups of rats was studied for the next 5 h. The maximal potassium level after loading occurred in both groups at 20-45 min.

The potassium concentration in the control rats then quickly returned to normal, but in the experimental animals it continued to remain at a high level (about 6.3 meq/liter).

Investigation of the way in which the effect of potassium retention in the plasma after potassium chloride loading depends on the dose of the factor showed that a dose of 0.05 μg has no action. With an increase in the dose from 0.1 to 5 μg the effect appreciably increased, and with a further increase, a phase of saturation occurred. Consequently, the factor has its physiological action in small doses, a characteristic feature of typical hormone-like substances.

The factor is evidently a specific component of the pineal gland. This is shown by the absence of hyperkaliemic activity in preparations obtained from the cerebral cortex and liver (Table 2). The cerebral cortex was chosen as a tissue most closely related to the pineal gland histologically, while liver tissue was chosen as a tissue with active metabolism. The specimens from the brain and liver were prepared in the same way as those from pineal tissue.

It is also clear from Table 2 that the activity of the factor isolated from the pineal gland is not due to the presence of melatonin as an impurity, for melatonin has no hyperkaliemic effect.

Proof of the protein-peptide nature of the hyperkaliemic factor from the pineal gland is given by the fact that its treatment with proteolytic enzymes (trypsin and pronase) for 18 h (with an enzyme : factor ratio of 1 : 50) completely abolished its activity.

The adrenal mineralocorticoids are known to regulate the electrolyte level in the body. It can therefore be postulated that the factor isolated from the pineal gland exerts its action indirectly through the adrenals. With this in mind tests were carried out to study the effect of the factor on the potassium concentration in adrenalectomized animals. The rats were adrenalectomized 5 days before the experiments. The material was injected into the rats in the same doses as in the previous experiments. The potassium concentration in the plasma was determined 5 h after potassium chloride loading (Table 3).

It is clear from Table 3 that adrenalectomy alone leads to an increase in the potassium concentration in the blood plasma, but the hyperkaliemic activity of the factor was also exhibited in adrenalectomized animals.

A physiologically active principle capable of influencing potassium metabolism in the body was thus isolated from the pineal gland. However the mechanism of its action is not yet clear. The hyperkaliemic effect of the factor may perhaps not be primary, but merely indirect evidence of its action at other levels. However, it should be noted that the factor isolated by the authors selectively changes the plasma potassium concentration and has no effect on the sodium and calcium concentrations.

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