SEASONAL CHANGES IN THE EFFECT OF MELATONIN ON THYROID FUNCTION

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It has recently been shown that variability of the peripheral action of melatonin is largely dependent on the rhythm of sensitivity of target tissues to it [3, 7]. Some works [4] postulate the "superposition" of two rhythms in this connection — the rhythm of melatonin production and the rhythm of sensitivity of target organs to it. Other workers [5, 6] consider that the sensitivity of target tissues to melatonin is regulated by melatonin itself, i.e., that realization of the effect of exogenous melatonin requires preservation of the source of the endogenous hormone, namely the pineal gland. Consideration of these data explains the information published previously, that injection of melatonin is effective only in the evening [2], for during the morning the receptors of the hormone are blocked by endogenous melatonin, secreted at night in large quantities. Thyroid activity is known to undergo considerable seasonal fluctuations, and it is accordingly interesting to study the sensitivity of the thyroid gland (TG) to the regulating influence of the pineal gland in different seasons of the year.

The aim of this investigation was to study changes in the response of the rat TG to injection of melatonin in the winter and summer.

EXPERIMENTAL METHODS

Experiments were carried out on 185 mature male Wistar rats kept under conditions of long (summer) and short (winter) daylight. The animals were kept on an ordinary diet of food and water, with natural alternation of day and night. The melatonin preparation (N. N. Suvorov, S. Ordhonikidze All-Union Pharmaceutical Chemical Research Institute) was injected in a dose of 1 μg/g body weight in 0.2 ml of physiological saline in the interval between 5 and 6 p.m. intraperitoneally for 5 and 10 days. Control animals received an injection of the same volume of physiological saline. The animals were decapitated under ether anesthesia on the 6th and 11th days respectively. Concentrations of tri-iodothyronine (T_3), thyrotoxin (T_4), and thyrotrophic hormone (TSH) in the blood plasma were determined by radioimmunoassay using test kits from Byk-Mallinckrodt (West Germany), and in some experiments the free thyroxine index (IFT_4) was determined by the method of saturation analysis. Uptake of ^{131}I by TG was determined by the usual method 24 h after injection of 3 μCi of ^{131}I into the animal.

EXPERIMENTAL RESULTS

Under conditions of long daylight, injection of melatonin for 5 days caused a decrease in the degree of ^{131}I uptake by TG by 28% and a small decrease in the plasma T_3 level (Table 1). Conversely, the blood T_4 level was significantly higher than in the control. The plasma TSH level was virtually indistinguishable from the control.

After injection of the preparation for 10 days a more marked decrease in the degree of ^{131}I uptake by the gland tissue was observed (by 37% compared with the control). Against the background of a plasma T_3 concentration which was lowered by the same degree as in the previous series of experiments, the T_4 level was the same as in the control. The main difference from the results of the previous series of experiments was an increase (Table 1) of 29% in the plasma TSH level of rats receiving melatonin.

It can be concluded from analysis of these data that after injection of melatonin for 5 days there is some decrease in the rate of extrathyroid conversion of T_4 into T_3 and a de-

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TABLE 1. Seasonal Differences in the Effect of Melatonin on Thyroid Activity in Rats

Experimental conditions	Summer						Winter					
	Number of animals	Duration of melatonin injections, days	^{131}I uptake by TG, %/mg TG tissue	plasma concentration of T_3, nmoles/liter	plasma concentration of T_4, nmoles/liter	plasma concentration of TSH, mU/liter	Number of animals	^{131}I uptake by TG, %/mg TG tissue	plasma concentration of T_3, nmoles/liter	plasma concentration of T_4, nmoles/liter	plasma concentration of TSH, mU/liter	IF T_4, relative units
Control	10	5	2,9±0,21 (7)	1,5±0,22	41±4,8	1,6±0,30	10	4,1±0,13 (10)	1,7±0,10	73±3,7	1,5±0,08	65,7±3,11
Melatonin	12		2,1±0,12* (10)	1,3±0,24	55±4,1*	1,7±0,12	12	3,6±0,16* (9)	1,3±0,11*	57±2,1*	1,1±0,09*	63,5±4,30
Control	9	10	3,8±0,10 (11)	1,4±0,10	54±5,8	1,4±0,11	10	4,7±0,20 (10)	1,5±0,13	74±3,7	1,4±0,09	66,6±3,92
Melatonin	13		2,4±0,08** (12)	1,2±0,16	63±7,1	1,8±0,14*	10	3,5±0,11** (10)	1,0±0,09*	57±4,1*	0,7±0,08**	45,6±2,17*

Legend. *P < 0.05, †P < 0.001 compared with control. Number of animals shown in parentheses.

crease in the degree of ^{131}I uptake by the gland, i.e., some inhibition of TG function. The absence of changes in the plasma TSH level at this stage of the investigation may be evidence of the parahypophyseal genesis of the changes observed.

The suggestions put forward are confirmed by analysis of the results of the experiments of series II, in which the increase in the plasma TSH concentration took place secondarily, evidently in connection with the prolonged fall of the T_3 level. Similar results with analogous conclusions were published previously [1]. In this connection the hypothesis of heterogeneity of sensitivity of target tissues to the action of melatonin will be recalled [5, 8]. The hypothalamus is one of the main targets for melatonin, and for that reason a decrease in its sensitivity to the action of melatonin may perhaps lie at the basis of the noninvolvement of the hypothalamo-hypophyseal axis in realization of the effect of melatonin on TG under conditions of long daylight. Meanwhile an effect of melatonin on the iodine-ingestive function of the gland and on the level of extrathyroid conversion of T_4 into T_3 is observed.

In winter (short daylight) injections of melatonin for 5 days caused changes in the same direction in all parameters studied (T_3, T_4, TSH). The T_3 level fell more definitely in this case than in summer (by 28 and 14% respectively), but the change in the plasma T_4 level showed a different trend (Table 1). IST_4 in the plasma of rats receiving melatonin was unchanged. The plasma TSH level was definitely lowered (by 27%).

Injection of melatonin for 10 days was accomplished by preservation of low blood T_4 levels, but against this background an appreciable decrease in IST_4 could be detected, evidence of a relative decrease in the free T_4 concentration in the plasma. The degree of ^{131}I uptake by the gland tissue and the blood T_3 level were lowered more demonstratively than in the previous series of experiments. The plasma TSH level was significantly lowered (Table 1).

The results obtained in the winter preriod thus differed significantly from those described above. The action of melatonin on TG under conditions of short daylight was one of definite inhibition of its function against the background of a progressive decrease, despite the low T_3 level, in the plasma TSH concentration. It can accordingly be postulated that under these conditons not only did melatonin have a more pronounced inhibitory effect than in summer, but this effect was realized differently, through the hypothalamo-hypophyseal axis. Returning to the arguments given above, it may be considered that in winter sensitivity of all stages of the realization of its effect on thyroid function to melatonin increases.

Data in the literature [3, 7] on rhythms of peripheral sensitivity to melatonin have been concerned with the antigonadal effects of the pineal gland. The results of the present investigation indicate that this rule also applies to pineal-thyroid inter-relations. It can thus be tentatively suggested that each target organ for melatonin has its own rhythm on sensitivity to influences of the pineal gland.

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ELECTRICAL PROPERTIES AND TRANSMEMBRANE IONIC CURRENT OF SINGLE SMOOTH MUSCLE CELLS

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The study of the electrophysiological properties of smooth muscles is beset with great technical and analytical difficulties, due to their distinctive structural features. The small size of smooth muscle cells (SMC), the presence of electrical connection between them, and also their contractile activity are a substantial handicap for the effective use of microelectrode research techniques. Meanwhile the good electrical connection between SMC does allow the sucrose gap technique to be used for the study of their electrophysiological properties. However, because of the disadvantages inherent in this method (interdiffusion of solutions flowing through adjacent compartments, the presence of diffusion potentials and of stray bypass circuits) the possibility of quantitative analysis of the results obtained by its use is substantially limited [1]. These factors, and also difficulties in creating adequate conditions for the investigation of transmembrane currents by the voltage clamp method on multicellular smooth-muscle preparations, have acted as a stimulus for the search for possible ways of utilizing single SMC in electrophysiological research. By now several investigations have been published in which attempts have been made to study passive and active electrical properties [7-9] and also transmembrane currents [11] of single SMC, with the use of intracellular microelectrodes.

This paper describes the results of such investigations, using a single suction micropipet [3], which has significant advantages over the microelectrode method [3, 4].

EXPERIMENTAL METHODS

Experiments were carried out on freshly prepared myocytes on the guinea pig taenia coli and ileum. To obtain isolated cells, small pieces of muscle tissue were kept for 10-15 min in calcium-free Krebs' solution, and then subjected to enzyme treatment (30 min, 36°C, pH 7.3, without mixing) in a solution containing (in mM): NaCl 120.4, KCl 5.9, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11.5, to which were added 1 mg/ml of collagenase (type I, from Sigma, USA), 1 mg/ml of bovine albumin, and 0.5 mg/ml of soy trypsin inhibitor. Pieces of tissue were then transferred into a solution with composition resembling that of "KB medium" [6] and containing (in mM): KCl 85, KH_2PO_4 30, $MgSO_4$ 5, Na_2ATP 5, creatine 5, glucose 20, EGTA 3 (the pH of the solution was adjusted to 7.2 with KOH). The pieces of tissue in this solution were repeatedly passed through a Pasteur pipet until a cell suspension was obtained. After 1 h a drop of suspension was added to an experimental chamber with a volume of 0.1 ml, located in the field of vision of an inverted microscope, and filled with Krebs' solution. After 3-5 min the myocytes easily adhered to the glass bottom, so that the chamber could be perfused with normal or testing Krebs' solution, previously filtered through a filter with pore diameter of 0.5 μ. Perfusion of the chamber was stopped during recording. The experiments were carried out at room temperature. The procedure of preparing and filling the glass micropipets, and also of obtaining high-ohmic contact with the membrane surface was identical with that described previously [3]. The micropipets used in the experiments had a resistance of 1-2 MΩ and were filled with a solution of the composition mentioned above ("KB medium").

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