several lymphoid organs, but not to other tissues. The finding that cortisol-induced blood eosinopenia is much less pronounced in cortisol-treated splenectomized animals than in intact cortisol-treated animals suggests that cortisol-induced blood eosinopenia is due, at least in part, to a migration of these cells to the spleen. The decrease in the number of tissue eosinophils in lymphoid tissues after adrenalectomy suggests that eosinophil migration to lymphoid organs may also occur in physiological conditions under the effect of endogenous levels of corticosteroid hormones.

The role of eosinophil leukocytes in lymphoid organs is unknown. Lymphoid organs seem to be target organs for cortisol. Specific receptors for cortisol have been described in rat thymus lymphocytes[15-17], as well as in other populations of lymphocytes[18]. Cortisol is known to inhibit glucose uptake and metabolism in thymus lymphocytes[19] and subsequently to decrease macromolecular synthesis[20]. Cortisol induces an involution of lymphoid organs[21] and a depression of the immune response[22]. Eosinophil leukocytes are thought to be involved in the immune mechanisms[23,24]. It is possible that the migration of eosinophil leukocytes to lymphoid organs under the effect of cortisol plays a role in the regulation of the immune reaction by cortisol. It is not known whether this migration is secondary to some of these conditions, to a release of an eosinophilotactic substance produced by T lymphocytes[25] or another eosinophilotactic factor, or is mediated by a direct influence on the cells, as was proposed to explain a specific eosinophil migration to other organs by other hormonal stimuli[14,26]. The possibility of trapping eosinophils in lymphoid tissue under the influence of glucocorticoids to remove these cells from the blood stream cannot be excluded. Further studies are necessary to elucidate the role of eosinophil leukocytes in the immune reaction.

Table 2. Effect of cortisol treatment and/or splenectomy on blood eosinophilia

Experimental condition	Number of eosinophils per mm^3 of blood ± SEM
Control rats	356.15 ± 50.22
Intact 6 h cortisol-treated rats	8.80 ± 5.39
Splenectomized untreated rats	574.17 ± 93.99
Splenectomized 6 h cortisol-treated rats	218.89 ± 40.28

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Effects of oestrogen and progesterone on rat pineal N-acetyl transferase activity and melatonin production

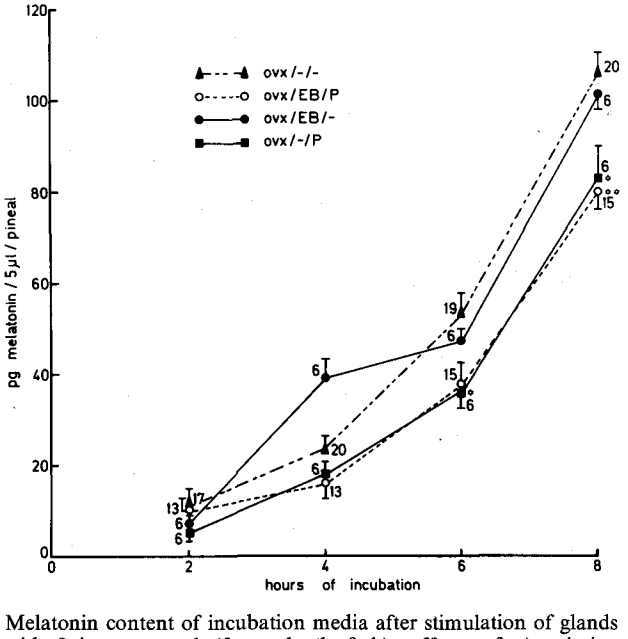
M. Wilkinson[1,2] and Josephine Arendt

Department of Physiology and Department of Pediatrics and Genetics, University of Geneva Faculty of Medicine, 1211 Geneva 4 (Switzerland), 24 October 1977

Summary. We have extended previous studies on pineal β-receptors to include effects of oestradiol or PMSG treatment in the immature female rat. Neither manipulation has any effect on norepinephrine-induced N-acetyl transferase (NAT) activity in vitro. In the adult ovariectomised rat oestrogen/progesterone priming exerts a small sensitising effect to β-stimulation with isoproterenol. Progesterone alone, in vitro, inhibits the release of melatonin from pineals of adult ovariectomised rats.

We have reported[3] that the sensitivity of pineal β-receptors is responsive to changes in hypothalamic function. During precocious sexual maturation after hypothalamic lesions the pineals of female rats are more sensitive to norepinephrine. The cause of this increase is obscure, though high plasma oestrogen titres could be responsible. We have extended previous work to look at the effects on β-receptor sensitivity of different methods of priming the immature female rat with oestrogen. Oestrogen titres were increased either by injection of oestradiol benzoate (OB) in oil or by pregnant mare serum gonadotrophin (PMSG), a treatment known[4] to elevate blood oestrogen. The influence of oestrogen treatment on pineal adenylyl cyclase has been described[5] in the adult rat. Thus oestrogen/progesterone inhibits norepinephrine-stimulated adenylyl cyclase in vitro, whilst Illnerová[6] found no effect of this treatment on isoproterenol-stimulated NAT activity in vivo. We have repeated the experiments of Weiss and Crayton[5] and have determined the effects of oestrogen and progesterone on pineal NAT after stimulation with a very low concentration[7] of isoproterenol (5×10^{-9} moles/l). In addition preliminary experiments show that progesterone inhibits the release of melatonin in vitro from pineals of ovariectomised rats.

Female rats (Sprague-Dawley SIV 50) were maintained under controlled lighting conditions (lights on 07.00–19.00 h). The rats were prepared for the experiments as follows: a) Animals were injected at 17.30 h on day 25 of life with either 20 µg OB (oil; s.c.) or oil and sacrificed the following morning (approximately 09.00 h). Pineals were dissected and placed in culture (see below). b) Animals were injected at 10.00 h on day 23 of life with PMSG (10 IU, s.c.; Gestyl®, Organon). Controls received vehicle only. Animals were sacrified at 09.00 h on day 25 ('pro-oestrus') or on day 26 ('oestrus') and pineals were placed in culture. c) Female rats (approximately 180 g) were ovariectomised 2 weeks before use. OB was injected 53, 29 and 5 h before sacrifice (50 µg/kg in oil, s.c.) and progesterone in oil (1 mg/kg, s.c.) 5 h before sacrifice[5]. Controls received oil only. Rats were killed at 14.00 h and pineals placed in culture. d) Female rats (approximately 200 g) were ovariectomised (10.00 h) and treated immediately with either OB (20 µg, in oil, s.c.) or oil. 48 h later the rats were sacrificed and pineals placed in culture in the presence or absence of progesterone (10 µg/ml). 4 groups of pineal cultures were obtained: 1. from ovariectomised animals (OVX); 2. from ovariectomised animals primed with OB (OVX/OB); 3. from ovariectomised animals cultured in the presence of progesterone (OVX/P); and 4. from ovariectomised animals, OB-primed, cultured in the presence of progesterone (OVX/OB/P). Glands were placed directly into a culture medium (see below) containing either L-norepinephrine (bitartrate, SIGMA; 10^{-6} moles/l) or L-isoproterenol (SIGMA; 5×10^{-9} moles/l). Pineals from groups a and b above were stimulated with norepinephrine (2 h) as previously described[3]. Groups c and d were stimulated with isoproterenol (8 h). Progesterone was added as a solution in propanediol (1% of final medium). Propanediol was added to control incubations at the same concentrations. 2–3 pineals were placed in 1 culture dish (60 × 15 mm; 1.5 ml of medium), except for the progesterone experiments in which each dish contained 1 pineal. Culture medium was 199 (GIBCO, catalogue No. BCL 193) supplemented with



Melatonin content of incubation media after stimulation of glands with L-isoproterenol (5 nmoles/l; 8 h); effects of a) priming ovariectomised animals with oestrogen and b) progesterone in vitro (10 µg/ml). Numbers refer to number of pineals; each point ±SEM ** $p < 0.01$; * $p \leq 0.05$. Groups OVX/P and OVX/OB/P are compared with OVX/OB.

sodium pyruvate (1 mmole/l), MEM non-essential amino acids (GIBCO; 1 ml of 100× concentration solution in 100 ml final medium), glucose (500 mg/100 ml) and ascorbic acid (0.1 mg/ml). Cultures were incubated at 37 °C under a water saturated atmosphere of 95% air/CO_2. Cultures were incubated at 37 °C under a water saturated atmosphere of 95% air/CO_2. Pineals were removed from culture and frozen at −30 °C. NAT activity was determined as before[3]. Culture medium from the progesterone experiments was removed as 100 µl aliquots at intervals of 2 h. Melatonin was determined by radioimmunoassay[8]. The medium was diluted in assay buffer and assayed without extraction. [^{3}H]-MT (S.A.: 26 Ci/mM) used as tracer was obtained from New England Nuclear Corporation. Statistical analysis was performed by Student's t-test.

A single injection of OB (20 µg) had no effect on the rise of NAT after stimulation with norepinephrine (10^{-6} moles/l; 3 h), when compared with controls. The respective figures, in p-moles of [^{14}C]-N-acetyl tryptamine formed per pineal per h are: 684±40 (9) (OB) and 663±60 (9). The numbers in brackets refer to the number of pineals. In the PMSG experiments pineals were removed from animals on the day preceding ovulation ('pro-oestrus') or on the day of ovulation itself. Again, no differences in the response of NAT were observed between treated animals and controls on 'pro-oestrus' or on the day of ovulation. The figures for treated and control groups respectively are pro-oestrus: 537±104 (5) and 609±45 (8); oestrus: 687±50 (9) and 632±65 (8) (in p-moles/gland h). The effectiveness of PMSG treatment was checked for the day preceding ovulation by dissecting and weighing uteri (91.0±4.3 mg versus 39.7±2.0 mg; $p < 0.001$) and for the day of ovulation by checking the fallopian tubes for the presence of ova. All the PMSG-treated animals (9/9) ovulated. In the Weiss and Crayton experiments[5] the results indicated that pineals from steroid-treated animals were slightly more responsive to stimulation than controls. However, only by pooling the results could a significant elevation of NAT activity over control values be obtained. The treated and control figures were as follows: 1332.5±104.9 (24) and 985.1±84.7 (23) ($p < 0.02$). The data shown in the figure illustrate the inhibitory effect of progesterone (10 µg/ml) on the release of melatonin. The effect of the steroid is exerted even in the absence of oestrogen priming. NAT activity was assayed at the end of the 8 h incubation period. The figures, in nmoles of N-acetyl tryptamine found per gland per h are respectively: OVX (1105±142; n = 17); OVX/OB (970±248; n = 6); OVX/OB/P (950±87; n = 13) and OVX/P (2194±174; n = 7).

Our results indicate that in the immature female rat, neither pharmacological doses of OB nor induced sexual maturation with PMSG are able to modify the response of pineal NAT to stimulation with norepinephrine in vitro. When compared with previous data[3] also using norepinephrine stimulation, these observations suggest that the effects on the pineal gland of precocious sexual maturation after PMSG treatment are different from those seen after similar induction with a hypothalamic lesion. We have proposed[9] that the effect of the lesion is mediated via oestrogen. However, since exogenous oestrogen appears to have no effect on pineal NAT in the present experiments, and since the high oestrogen titers produced by PMSG also have no effect, it is probable that the brain lesion-induced increase in pineal β-receptor sensitivity is mediated via another, unknown, mechanism. The results on the lack of an effect of oestrogen on NAT activation led us to reconsider the inhibition described by Weiss and Crayton[5] on pineal adenylyl cyclase activity. An effect of oestrogen on NAT induction has never been demonstrated[6,10,11]. The difficulties inherent in explaining this are therefore compounded

by our present data showing a small stimulatory, rather than inhibitory, effect of oestrogen-progesterone treatment on isoproterenol-induced NAT. The very low doses of isoproterenol (5 nmoles/l) which we have used when compared with the stimulatory dose of norepinephrine (10^{-4} moles/l)[5] could have different responses. Though in our hands, this concentration of norepinephrine gave identical results (in terms of NAT activation) in both treated and control animals (results not shown).

We attempted to study the role of progesterone in the above experiments by isolating its effect in vitro, i.e. by the addition of progesterone to cultures of pineals. Our preliminary experiments show clear differences in melatonin production when the culture media were assayed following stimulation. An analysis of the time course of this effect showed that progesterone exerts an inhibition of melatonin output (figure). A reduction in activity of pineal hydroxyindole-O-methyl transferase (HIOMT), the enzyme necessary for melatonin biosynthesis has been reported[12] in castrate female rats, chronically treated with progesterone. Also, it is known[13] that in the female rat HIOMT activity is depressed during pseudo-pregnancy, a period when progesterone levels are high. Experiments are in progress to further investigate, in terms of melatonin secretion, the effects of different concentrations of oestrogen/progesterone.

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2 Reprint requests should be addressed to M. Wilkinson, Department of Physiology, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP (England).
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HLA BW54 and B5 in Japanese diabetics with juvenile-onset and insulin-dependency (with special reference to the family history)

A. Kawa, M. Nakazawa, Y. Kono, S. Sakaguchi, S. Nakamura and T. Kanehisa

The First Department of Internal Medicine, Kagoshima University Medical School, Kagoshima 890 (Japan), 13 October 1977

Summary. The frequency of HLA BW54 and B5 in Japanese patients with JOD is increased and decreased, respectively. In JOD patients without a family history of MOD, the frequency of BW54 is significantly increased, whereas in JOD patients with a positive family history the frequency was not increased in a statistically significant manner.

There are some indications of a relationship between HLA, which is hereditary, and various diseases, including neoplastic diseases, viral infections and conditions with abnormal immune responses[1]. In recent publications the evidence that viral infection may be involved in the pathogenesis of juvenile-onset diabetes with insulin-dependency (JOD) has been presented[2,3]. Abnormalities in cellular immunity are also reported[4,5].

These findings suggest that there might be a possible relationship between at least some forms of diabetes mellitus and HLA. The increased frequency of HLA-B8 and/or BW15 in JOD among Caucasians was reported[6-9]. We reported a significant increase of BW54, previous J-1 or BW22-2, and a decrease of B5 in Japanese patients with JOD[10,11].

Diabetes mellitus is generally regarded as a genetically predisposed disease[12]. This appears to be obvious in maturity-onset diabetes (MOD)[13]. But the genetic contribution, as judged by family histories, to JOD seems to be less clear, because only less than half of the patients examined have a family history of diabetes mellitus[13,14]. JOD might be related to the genetic susceptibility to viral infection and/or abnormal immune responses.

In this study 27 Japanese patients with JOD were HLA typed according to NIH method[15], and these results were also compared to the absence or presence of a positive family history of MOD; 106 healthy subjects were also HLA typed as a control. All the subjects were typed for antigens A1, A2, A3, A9, A10, B5, B7, B8, B12, B13, B14, BW16, BW17, BW21, BW22, BW27, BW35, BW37, BW40 and BW54.

As clearly seen in table 1, JOD among Japanese is associated with an increased frequency of of BW54 (corrected $p < 0.002$) and a decreased frequency of B5 (corrected $p < 0.002$).

Our observation also revealed an increased frequency of BW35 and a decreased frequency of B5 in Japanese patients with Graves' disease[16]. Thus a decreased frequency in B5 appears to be a common characteristic in HLA phenotypes among Japanese patients with Graves' disease and JOD, while these two diseases among Japanese do not share, as far as an increased frequency is concerned, the

Table 1. Phenotype frequencies of HLA in Japanese JOD patients

	BW54		B5		B12	
	Control	JOD	Control	JOD	Control	JOD
(+)	22	17	58	3	8	1
(−)	84	10	48	24	98	22
χ^2	16.5		14.8		0	
p	0.0001		0.0001		NS	
Corrected p	0.0019		0.0019			

Table 2. Relation between BW54 and JOD with special reference to absence or presence of a family history of maturity-onset diabetes

	Control	Family history (−)	(+)
(+)	22	12	5
(−)	84	3	7
χ^2		20.1	1.4
p		0.0001	NS
Corrected p		0.0019	