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Tissue-specific modulation of rat glucocorticoid receptor binding activity by melatonin

St. P. Persengiev* and I. I. Kondova

Department of Immunoneuroendocrinology, Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, 1113 Sofia (Bulgaria)
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Abstract. The effect of melatonin and 2-Iodomelatonin on nuclear and cytosolic glucocorticoid receptors in the brain, pituitary, thymus and liver has been examined. The results indicate that both melatonin and 2-Iodomelatonin administration is associated with marked changes in the density and the affinity of cytosolic and nuclear forms of glucocorticoid receptors. These observations are discussed in the context of a possible involvement of pineal melatonin in the mechanisms regulating the behaviour and metabolism of steroid receptors.
Key words. Melatonin; 2-Iodomelatonin; glucocorticoid receptors; brain; thymus; liver; pituitary.

Glucocorticoids, acting via intracellular receptors, play an important role in maintaining the internal milieu and are used for a wide range of therapeutic purposes. Upon binding to a cognate hormonal ligand, the glucocorticoid receptors (GR) associate with specific DNA sequences, termed glucocorticoid response elements (GRE), and regulate specific gene expression[1]. The molelcular mechanisms for signal transduction and transcriptional regulation by the reactor remain to be determined, but it is apparent that the receptor interacts with the hormone, DNA and non-receptor protein factors. It is thus of the utmost importance to elucidate the factors and mechanisms regulating glucocorticoid receptor (GR) expression if the physiological and therapeutic actions of glucocorticoids are to be completely understood.

Previously, we have shown that chronic melatonin treatment differentially affects the activity of glucocorticoid receptors in the brain, pituitary and thymus[2–4]. The influence of the hormone was apparent when the steroid environment was subject to variation: the properties of glucocorticoid receptors were affected only in the presence of normal and increased systemic corticosterone concentrations, and not in adrenalectomized animals[5]. However, the biological significance of the effect of melatonin, and the mechanism by which it affects the receptors, remain obscure. The aim of the present study was to investigate the effect of endogenous melatonin and its synthetic analogue 2-Iodomelatonin on glucocorticoid receptors in two brain regions (hippocampus and hypothalamus), pituitary, thymus gland and liver. To define more clearly the mechanism of melatonin action both the cytosolic and nuclear forms of glucocorticoid receptors were analyzed.

Materials and methods

Adult male Wistar rats weighing 160–180 g were used. The animals were housed in groups of ten under controlled illumination (LD 14:10 h), with free access to standard lab chow and tap water. After 4 weeks of adaptation animals were divided into separate groups as follows: (a) controls; (b) melatonin treated; and (c) 2-Iodomelatonin treated. Hormonal treatment was provided for 6 days according to the following schedule: melatonin and 2-Iodomelatonin were dissolved in a minimal volume of ethanol, diluted in saline and applied once daily in doses of 100 µg/kg b.wt and 10 µg/kg b.wt respectively. The hormones were injected subcutaneously 4 h before the onset of darkness. Subjects of the corresponding control group received daily saline injections.

After 6 days of treatment the rats were sacrified and the hippocampus, hypothalamus, pituitary, thymus gland and liver were rapidly removed and stored at −80 °C until use. At the time of an assay the frozen tissues were thawed and homogenized with a Ultra-Turax homogenizer in 10 mM Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose and 10% glycerol. The nuclei were pelleted at 800 g, washed and resuspended in the same buffer containing additionally 1 mM EDTA, 2 mM EGTA, 0.1 mM DTT, 1 mM $MgSO_4$ and 1 mM $CaCl_2$. The initial supernatants were recentrifuged at 105,000 g for 1 hour at 4 °C in the same buffer containing additionally 0.1 mM DTT, 1 mM EDTA and 10 mM sodium molybdate, and the high-speed cytosol collected. The nuclear and cytosolic protein contents were determined[5a] and adjusted to 3.0–4.0 mg/ml.

Unoccupied nuclear glucocorticoid receptors were assayed by incubating 200 µl nuclear suspension with 50 µl ^{3}H-corticosterone in graduated concentrations (1.0–4.0 nM) for 2 h at 4 °C. The nuclei were then separated at 800 g, washed three times with ice-cold buffer and the pellet was extracted with ethanol. The unoccupied cytosol receptors were determined by the methods previously described.[6,7] In summary, 100 µl cytosol was incubated with serial dilutions of ^{3}H-corti-

costerone (0.5–20 nM) at 4 °C for 2 h and then separated with 500 µl dextran-coated charcoal at 800 g. Non-specific binding of both receptor analyses was carried out in the presence of a 500-fold excess of unlabelled corticosterone and a 100-fold excess of RU 38486 (Roussel-UCLAF, France). The EBDA/LIGAND program was applied for initial data analysis of the results from saturation experiments and subsequent mathematical selection of the appropriate model[8,9]. The results were analyzed for differences by analysis of covariances.

Results and discussion

In earlier work we showed that glucocorticoid receptors in the brain and thymus gland are affected by exogenously applied melatonin, which indicated that the steroid receptor system is one of the probable target sites of melatonin action[2–4]. The results of the present investigation indicate that daily administration of melatonin for six consecutive days considerably affected the biochemical properties of glucocorticoid receptors in all tissues studied (table 1, fig. 1 and 2). Scatchard analyses of unoccupied cytosol and nuclear receptors showed significant differences in the binding activity of receptor forms. The administration of melatonin was associated with dramatic changes in the density and the affinity of the cytosolic and nuclear components of glucocorticoid receptors. Treatment with the potent melatonin analogue 2-Iodomelatonin duplicated the effect of melatonin in all cases. 2-Iodomelatonin was active when administered in a dose 10-fold less than that of melatonin, which is in accordance with the previous findings concerning its activity[10–12].

The influence of melatonin on the glucocorticoid receptors was not equal in the different target tissues. This led us to divide the tissues into three groups based on the results from the in vitro binding studies of cytosol and nuclear receptor distribution after melatonin administration (see table 1). In the first group were the hypothalamus and pituitary, in which the apparent decrease in the affinity and increase in the capacity of cytosol receptors were associated with an increase in the affinity and decrease in the capacity of nuclear receptor molecules. The hippocampus represented the second group, where the effect of melatonin was the same on both the nuclear and cytosolic receptors and was restricted only to an increase in the Kd constant. The effect of melatonin on steroid receptors in the thymus and liver was opposite to that observed in the hypothalamus and pituitary, and therefore these tissues were characterized as a third group in the present study. The biological significance of the data reported herein remain unclear, but the observations by other authors, that melatonin alters the activity of estrogen and androgen receptor systems as well[13–16] suggest that the steroid receptor superfamily is a probable target of melatonin action.

Table 1. Biochemical characteristics of cytosolic and nuclear glucocorticoid receptors in different tissues after treatment with melatonin and 2-Iodomelatonin. Kd (nM) — dissociation constant; Bmax (fmol/mg protein) — number of binding sites; each group represents the results of two independent determinations. *$p < 0.05$ vs control group.

Treatment Group	Cytosolic binding Kd	Bmax	Nuclear binding Kd	Bmax
Group I Hypothalamus				
Vehicle	8.8 + 3	16.5 + 5.8	27.3 + 6	60.3 + 11
Melatonin	24.9 + 2*	51.1 + 9.5*	17.5 + 6	17.2 + 5*
2-Iodomelatonin	16.2 + 5*	48.2 + 10.2*	2.2 + 1*	8.3 + 3*
Pituitary				
Vehicle	2.2 + 1	7.2 + 3.4	131 + 25	53.4 + 14
Melatonin	11.4 + 7*	22.3 + 9.6	62.8 + 9*	38.4 + 15
2-Iodomelatonin	10.5 + 3*	16.6 + 4.5	41.6 + 9*	17.7 + 6*
Group II Hippocampus				
Vehicle	4.8 + 1.4	17.2 + 6.1	8.8 + 2	8.6 + 3
Melatonin	12.3 + 3.4*	17.2 + 5.3	31.1 + 8*	18.3 + 6*
2-Iodomelatonin	8.6 + 2*	19.7 + 4.2	15.2 + 4*	7.1 + 4
Group III Thymus				
Vehicle	196 + 86	175 + 43	6.7 + 2	17.1 + 7
Melatonin	31.4 + 12*	36.2 + 8*	18.2 + 5*	3.4 + 1*
2-Iodomelatonin	48.8 + 21*	49.5 + 12*	15.4 + 7	7.3 + 4
Hepar				
Vehicle	214 + 87	220 + 66	4.3 + 2	2.8 + 3
Melatonin	96.6 + 33*	72.3 + 23*	10.9 + 3*	6.8 + 4
2-Iodomelatonin	120 + 54*	84.8 + 43*	8.8 + 2*	4.8 + 2

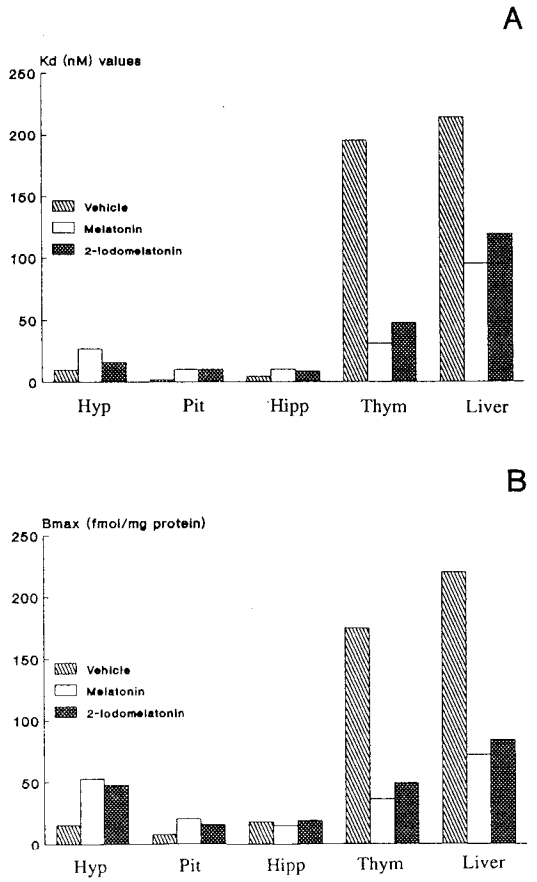

Figure 1. Graphical representation of the changes in the biochemical properties of cytosolic glucocorticoid receptors following melatonin and 2-Iodomelatonin treatments. Hyp—hypothalamus; Pit—pituitary; Hipp—hippocampus; Thym—thymus.

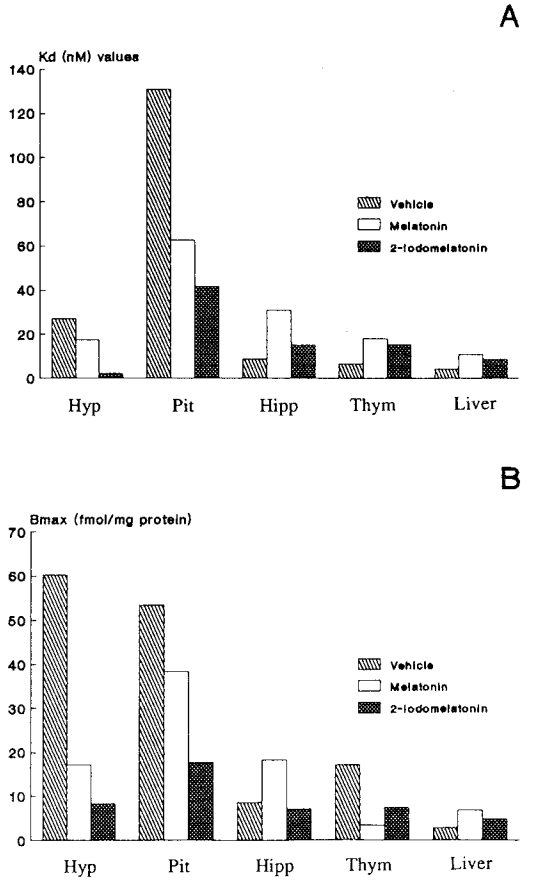

Figure 2. Graphical representation of the changes in the biochemical properties of nuclear glucocorticoid receptors following melatonin and 2-Iodomelatonin treatments. Hyp—hypothalamus; Pit—pituitary; Hipp—hippocampus; Thym—thymus.

Our finding that the biochemical properties of a major steroid hormone receptor may be affected by another hormone under supraphysiological conditions may provide further insight into the factors and mechanisms regulating the behaviour and metabolism of these receptor proteins. The mode of melatonin action remains obscure and subject to speculation, but there are, it would seem, at least three possible mechanisms to explain this action: (1) Melatonin could influence net accumulation of GR by altering GR gene expression or subsequent mRNA processing. (2) It could act directly on the GR to induce conformational changes, activating or inhibiting otherwise discrete functional domains. (3) It could promote the synthesis of intermediate factors which in turn induce modification of GR binding activity. On the other hand, the alteration of GR activity by melatonin may also have important therapeutic and biochemical implications and further studies should clarify this issue.

* Address for correspondence: St. P. Persengiev, DVM, Ph.D., Neurobiology Group, Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545, USA

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