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Effects of Freund's complete adjuvant on the diurnal rhythms of neuroendocrine processes and ornithine decarboxylase activity in various tissues of male rats

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Abstract. The aim of this study was to investigate the effects of Freund's complete adjuvant (FCA) on the diurnal rhythms of hormonal parameters in serum and ornithine decarboxylase (ODC) activity in various tissues of male rats. On days 1–2 after FCA, increase of ODC activity (used to evaluate the level of activation) was observed in the hypothalamus, pituitary gland, adrenal medulla, adrenal cortex, liver and lymphoid tissues, while the ODC activity in the kidney was reduced. This was accompanied by an increase in serum corticosterone. On days 3–4 after FCA, ODC activity remained elevated in the pituitary gland, liver and lymphoid tissues, while the ODC activity in the testes and pancreas was reduced; kidney ODC activity returned to baseline. This was associated with increased serum levels of prolactin (Prl) and luteinizing hormone, but decreased growth hormone, testosterone and insulin. The increase in ODC activity in the thymus, as well as the reduced ODC activity in the testes and kidney, can be obtained with paraffin. Furthermore, bromocryptine microcapsules (CBLA) reduced the FCA-induced increase of ODC activity in the pituitary gland, liver and lymphoid tissues (days 3–4) but did not affect the changes in other tissues. The increase in ODC activity in the pituitary gland, liver and lymphoid tissues is specific for FCA. A role for Prl in the induction of ODC in liver and lymphoid tissues is suggested by the fact that CBLA suppresses this enhancement.
Key words. Freund's complete adjuvant; diurnal rhythms; ornithine decarboxylase; prolactin.

Dysfunctional communication between the neuroendocrine and immune systems appears to contribute to the development of autoimmune diseases in rodents, chickens and humans [1, 2]. In rats, FCA induces an arthritic disease within 10 days, preceded by multiple neuroendocrine alterations. This is reflected in the early phase (i.e. before the development of adjuvant arthritis [AA]) by the increased production of pituitary hormones, e.g. Prl, luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH) [3–5].

Ornithine decarboxylase (ODC) activity can be used for the assessment of the degree of activation of the various organs involved [6]. Mitogens cause a rapid stimulation of ODC activity in lymphocytes [7]. The very short half-life of ODC and the speed with which it responds to regulatory stimuli can be exploited to observe diurnal phases of physiological events. Thus, diurnal rhythms of ODC activity have been reported in mouse liver [8], rat pancreas [9], and rat pituitary gland, adrenal cortex and lymphoid tissues [3–5]. The consideration of diurnal variations allows certain relationships to be recognized. These rhythms can be due to various factors, including neuroendocrine fluctuations. For instance, regulation of ODC activity by Prl has been shown for the mammary gland [10], testis [11], seminal vesicles [12], hepatocytes [13], pancreas beta-cells [14], lymphoid tissues [3–5, 15] and Nb2 T-lymphoma cells [16]. In order to explore the possibility that early neuroendocrine events are associated with the development of AA, we decided to measure the diurnal rhythm of ODC activity in various tissues taken from FCA-treated rats. It can be argued that the possible relationships between modulation of ODC activity during the latency period after FCA and the development of arthritis is uncertain. On the other hand, using the cyclooxygenase inhibitor indomethacin, an early study [17] reported that it is sufficient to block some processes occurring at the time of FCA injection, and the effect of such blockade is still evident 2 weeks later. This may be due to immunomodulation associated with antigen recognition processes [18].

Prl is an immunostimulatory hormone [1, 19], and inhibition of its secretion by the dopamine agonist bromocryptine reduces the incidence and severity of AA [3, 20]. The blocking of increased production of Prl in the early latency phase after FCA reduces AA. In mice, the immunoregulatory effects of Prl and bromocryptine seem to be time-of-day dependent [21]. We observed the effect of bromocryptine microcapsules (CBLA) on ODC activity during the dark phase, i.e. during the peak of FCA-induced Prl production [3, 5]. The differential effect of the drug could provide information about the organ systems involved in the development of AA.

Materials and methods

Male Sprague-Dawley rats aged 3 months were injected with 0.2 ml of water-paraffin emulsion (1:10, vol/vol)

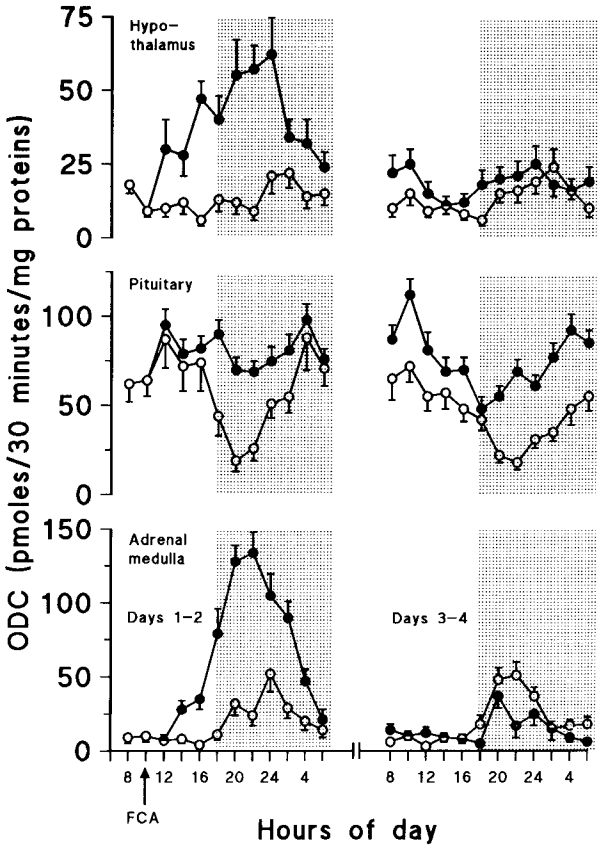

Figure 1. Diurnal rhythms of ODC activity in the hypothalamus, pituitary gland and adrenal medulla in male rats aged 3 months (○) and in animals treated with FCA (● 0.5 mg/rat of *M. butyricum*) (mean ± SD, n = 6 per time point). The paraffin-treated rats are not shown. The shadow represents the dark phase. On days 1–2 upon FCA, increased hypothalamus ODC activity is associated with enhanced pituitary and adrenomedullary ODC activities. On days 3–4, pituitary ODC activity remains elevated.

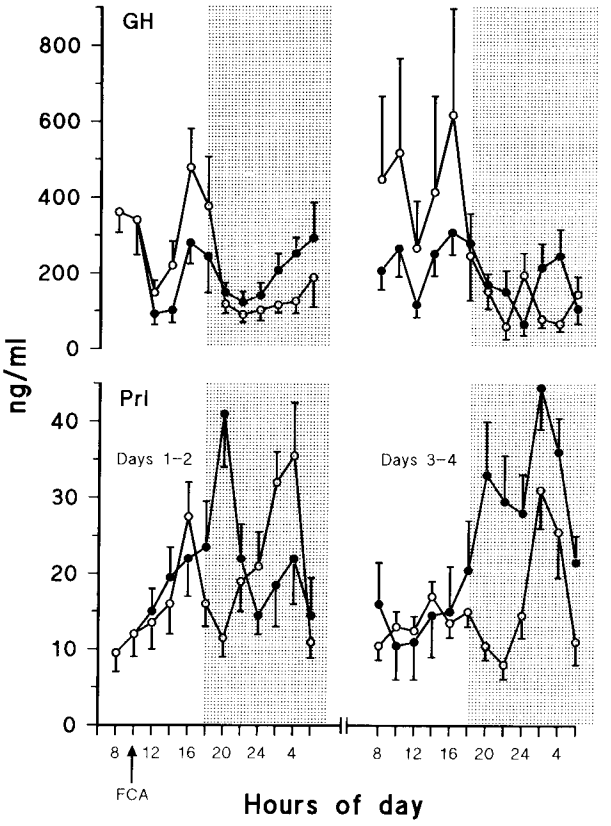

Figure 2. Diurnal rhythms of serum GH and Prl following injection of vehicle or FCA in male rats aged 3 months. Same legend as in figure 1. After FCA, the level of GH is decreased, while Prl is increased during the dark phase.

or with FCA (emulsion containing 0.5 mg of *Mycobacterium butyricum*, Difco, Detroit, MI) i.d. at base of the tail (day 1). One group of rats was pre-treated with CBLA (1 mg/kg i.m., Parlodel LA, Sandoz, Basel, on day 2). Control rats and those receiving FCA only were pre-treated with placebo microcapsules. The rats were pair-housed in a temperature (24 ± 1 °C)- and light (12 h/day)-controlled room. Food and water were given ad libitum [3].

The experiment was repeated three times within 3 months. Each time, two animals per group were killed by decapitation every 2 h on days 1–2 and 3–4. Arthritis would appear on days 9–10. Trunk blood was collected and various tissues were frozen in liquid nitrogen. Serum was kept at −20 °C and tissues at −80 °C until examination. The results of the three series were pooled.

The serum levels of hormones were estimated by radioimmunoassay (done at Sandoz, Basel [4]).

ODC activity was determined as reported elsewhere [3]. Briefly, tissues were homogenized and centrifuged, and the supernatant fractions were incubated for 30 min at 37 °C in glass tubes—fitted with rubber stoppers and center wells each containing a filter paper—in the presence of [1-^{14}C]ornithine hydrochloride (Amersham, Arlington Heights, IL). The $^{14}CO_2$ liberated from the enzymatic reaction was collected on the filter papers, and radioactivity was counted in Toluol/Omnifluor scintillant (New England Nuclear, Boston, MA). Protein concentrations in homogenates were measured spectrophotometrically at 595 nm using Bio-Rad dye reagents (Bio-Rad, Richmond, CA).

Data in the tables and figures are expressed as the mean ± standard deviation (SD). Means over 12 h or 24 h considered all animals receiving the same treatment. The Mann-Whitney U-test was used for comparison of medians. The Spearman's rank correlation was employed as appropriate; $p < 0.05$ was taken as the level of significance.

Results

Diurnal rhythms of the hypothalamic-pituitary and -adrenomedullary axis. Table 1 summarizes our findings concerning the diurnal rhythms of ODC activity in various tissues in the male rat and the influence of Freund's incomplete (paraffin oil) or complete adjuvant. Figure 1 illustrates these rhythms in the hypotha-

Table 1. Summary of the diurnal rhythm of ODC activity in the male rat. The tissues are divided into two groups with peaks either during the light (A) or dark phase (B). Influence of Freund's incomplete (paraffin oil) or complete adjuvant (FCA) during the light-dark cycle on days 3–4 after treatment, compared with untreated control rats.

	Affected during the light phase by		Affected during the dark phase by	
	paraffin oil	FCA	paraffin oil	FCA
A. Maximum during the light phase (06.00–18.00 h)				
Pituitary gland	=	++	=	+++
Thymus	+	++	+	+++
Bone marrow	=	+++	=	++
Lymph nodes	=	++	=	++
Spleen	=	++	=	++
Lung	++	++	++	++
Heart	=	=	=	=
Testes	--	--	--	--
B. Maximum during the dark phase (18.00–06.00 h)				
Hypothalamus	=	+	=	+
Liver	=	+	=	++
Adrenal cortex	=	++	=	-
Adrenal medulla	=	=	-	---
Pancreas	-	+	---	---
Kidney	=	--	=	-

---: 60 to 40% decrease, --: 40 to 20% decrease, -: 20 to 10% decrease, =: no change (−10 to +10%), +: 10 to 30% increase, ++: 30 to 60% increase, +++: more than 60% increase.

lamus, anterior pituitary gland and adrenal medulla (the weak effect of paraffin alone is omitted); figure 2 shows the diurnal rhythms of growth hormone (GH) and Prl.

Hypothalamic ODC showed enhanced activity during the dark phase. In contrast, ODC activity in the pituitary gland showed an increase during the morning, while the minimum occurred at the beginning of the dark phase. The serum level of GH was elevated during the light phase with a transient decrease in the afternoon (fig. 2), whereas the serum level of Prl was increased during the dark phase.

In controls (with placebo microcapsules i.m.) and in paraffin-treated rats, mean hypothalamic ODC activity over 24 h (on days 3–4 on paraffin) was 47 ± 15 and 48 ± 18 pmol/30 min/mg protein, respectively. Similarly, no significant changes over 24 h were found after paraffin alone for pituitary ODC activity, or for the mean serum levels of GH and Prl (data not shown). After paraffin, a weak reduction of adrenal medulla ODC activity could occur during the dark phase (table 1). However, in general, the injection of paraffin alone has no effect on these parameters.

Activation of the hypothalamic-pituitary and -adrenomedullary axis upon FCA. After FCA, the hypothalamic ODC activity significantly increased on days 1–2 (during both light and dark phases), but probably not thereafter. The change in adrenal medulla ODC activity was very similar, with the exception of an important reduction during the dark phase on days 3–4.

On days 1–2, pituitary ODC activity showed the characteristic decrease at the beginning of the dark phase. On days 3–4, it remained increased over the baseline. Mean pituitary ODC activity over 24 h, on days 3–4, reached 101 ± 24 pmol/30 min/mg protein ($p < 0.001$ compared with 55 ± 18 pmol/30 min/mg in controls). The diurnal rhythmicity of GH was partially lost. On days 3–4, an additional increase in Prl was obvious during the dark phase; the mean serum level of Prl over 24 h on days 3–4 reached 21 ± 10 ng/ml ($p < 0.001$ compared with 14 ± 8 ng/ml in controls or 15 ± 7 ng/ml in paraffin-treated rats).

Diurnal rhythms of the hypothalamic-adrenocortical axis. Figure 3 presents the serum levels of ACTH and corticosterone (CS), as well as the diurnal rhythm of adrenal cortex ODC activity. An elevation of ACTH secretion can be observed during the dark phase, despite important interindividual fluctuations. Both adrenocortical ODC activity and serum concentration of CS showed maximal levels during the dark phase. In controls, a triangular relation can be observed between elevation of ACTH, enhanced adrenocortical ODC activity and increased CS release (r between 0.40 and 0.45, $p < 0.05$). In fact, the serum level of CS is highly significantly correlated with the elevation of adrenocortical ODC activity measured 2 h later ($r = 0.72$, $p < 0.001$). In controls and paraffin-treated rats, the mean level of ACTH over 24 h was 85 ± 42 and 109 ± 48 pg/ml, respectively (not significantly different). Similarly, the injection of paraffin alone did not affect the adrenocortical ODC activity or the serum level of CS (data not shown).

Activation of the hypothalamic-adrenocortical axis upon FCA. After FCA, on days 1–2, both adrenocortical

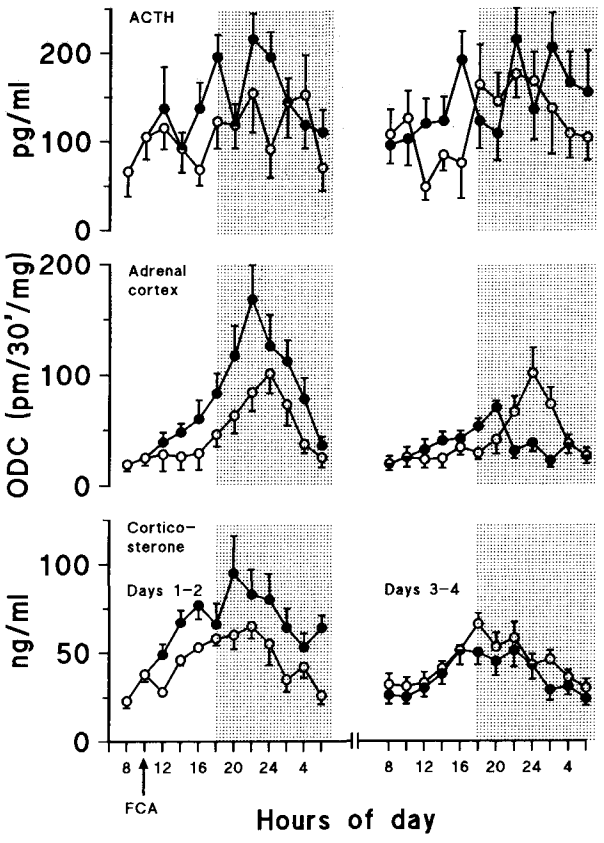

Figure 3. Diurnal rhythms of serum ACTH, ODC activity in the adrenal cortex and serum CS following injection of vehicle or FCA in male rats aged 3 months. Same legend as in figure 1. On days 1–2 upon FCA, the level of ACTH shows high fluctuations, while adrenal cortex ODC activity and the level of CS are further increased during the dark phase. On days 3–4, adrenal cortex ODC activity and CS return to baseline values.

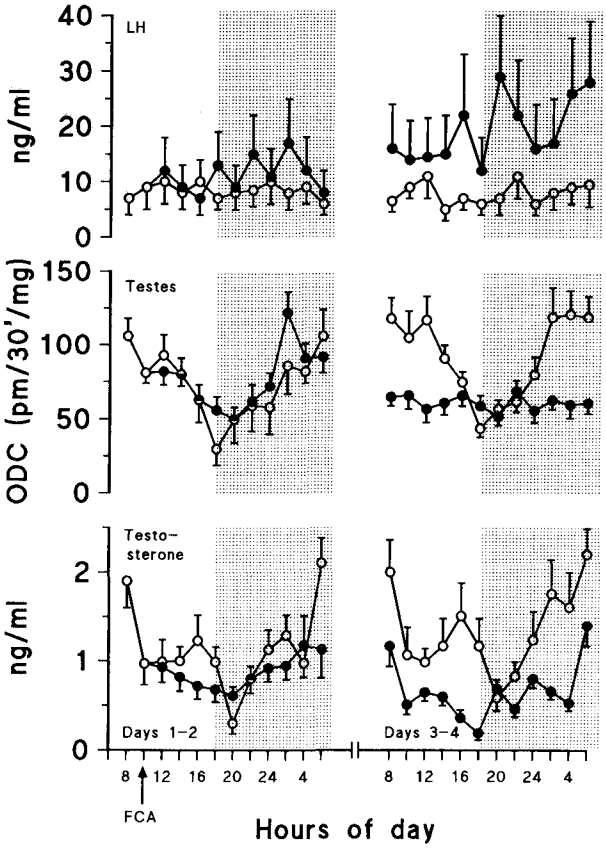

Figure 4. Diurnal rhythms of serum LH, ODC activity in the testes and serum testosterone following injection of vehicle or FCA in rats aged 3 months. Same legend as in figure 1. On days 3–4 upon FCA, the serum level of LH increases, while the diurnal rhythmicity of testes ODC activity is lost, and testosterone concentration decreases.

ODC activity and CS release were increased. On days 3–4, these parameters returned to baseline; the diurnal rhythms of ACTH and adrenocortical ODC activity were disturbed, the latter showing a relative increase during the light phase but a decrease during the dark phase.

Diurnal rhythms of the hypothalamic-gonadal axis. Figure 4 shows the serum levels of LH and testosterone, as well as the diurnal rhythm of testes ODC activity. The serum level of LH showed no diurnal rhythmicity (mean over 24 h: 50 ± 17 and 51 ± 11 ng/ml in controls and paraffin-treated rats, respectively). In contrast, at the end of the dark phase and the beginning of the light phase, testes ODC activity was enhanced, and the serum level of testosterone was elevated (means over 24 h: 108 ± 36 pmol/30 min/mg protein and 1.4 ± 0.5 ng/ml, respectively). Enhanced ODC activity and increased serum level of testosterone were correlated ($r = 0.72$, $p < 0.001$).

In controls and paraffin-treated rats, the mean level of LH over 24 h was 85 ± 42 and 109 ± 48 ng/ml, respectively (no significant difference). In contrast, the injection of paraffin affected testes ODC activity and the serum level of testosterone (means over 24 h on days 3–4: 54 ± 14 pmol/30 min/mg and 0.9 ± 0.4 ng/ml, respectively; $p < 0.05$ for both compared with controls).

Modulation of the hypothalamic-gonadal axis upon FCA. After FCA, on days 3–4, the level of LH was significantly increased (mean over 24 h: 69 ± 18 ng/ml, $p < 0.005$ compared with controls or paraffin-treated rats [above]). However, the diurnal rhythmicity of testes ODC activity was lost, and testosterone release was reduced (means over 24 h: 62 ± 11 pmol/30 min/mg protein and 0.7 ± 0.3 ng/ml, respectively; $p < 0.01$ for both). The findings for testes ODC activity and testosterone level resembled those observed with paraffin alone.

Diurnal rhythms of kidney, liver and pancreas ODC activity. Figure 5 presents the diurnal rhythms of ODC activity in the kidney, liver and pancreas. In controls, ODC activity in kidney, liver and pancreas, as well as the serum level of insulin (data not shown), were increased during the dark phase. Compared with controls, paraffin-treated rats showed decreased kidney ODC ac-

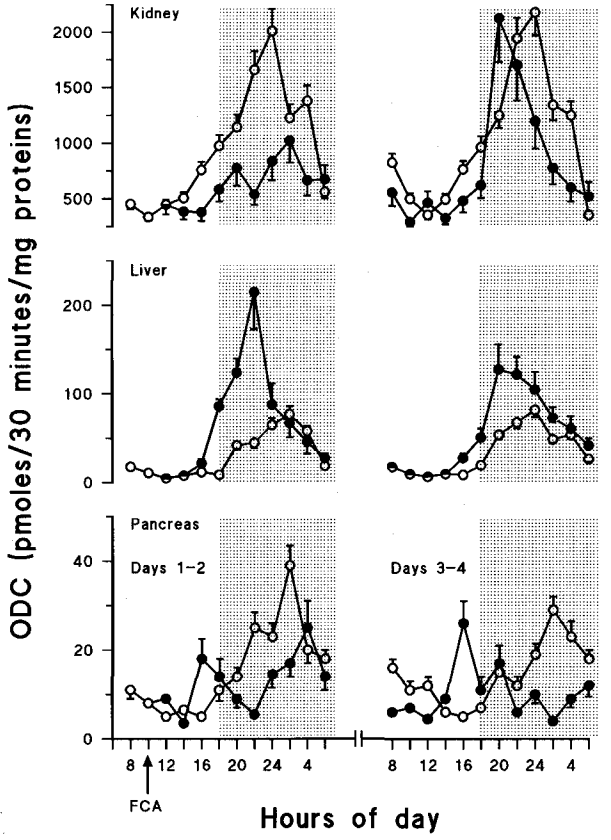

Figure 5. Diurnal rhythms of ODC activity in the kidney, liver and pancreas following injection of vehicle or FCA in male rats aged 3 months. Same legend as in figure 1. On days 1–2 upon FCA, kidney ODC activity is decreased, liver ODC activity is increased and the diurnal rhythmicity of pancreas ODC activity is partially lost. On days 3–4, ODC activity is restored in the kidney and remains elevated in the liver.

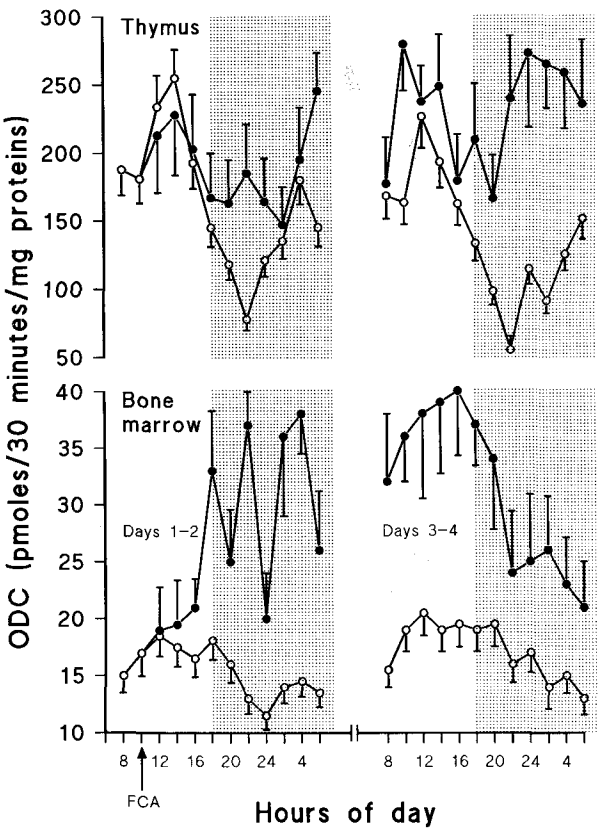

Figure 6. Diurnal rhythms of ODC activity in primary lymphoid tissues following injection of vehicle or FCA in male rats aged 3 months. Same legend as in figure 1. ODC activity is enhanced, but the diurnal rhythmicity is maintained.

tivity on days 1–2 (means for the next 24 h: 102 ± 46 and 68 ± 45 pmol/30 min/mg protein, $p < 0.01$). On days 3–4 after paraffin alone, decreased pancreas ODC activity (means over 24 h: 19 ± 6 and 11 ± 7 pmol/30 min/mg in untreated controls and paraffin-treated rats, respectively, $p < 0.05$) and reduced insulin secretion occurred (means over 24 h: 56 ± 16 and 38 ± 18 mU/ml, respectively, $p < 0.01$). Reduced pancreas ODC activity was more obvious during the dark phase (table 1). Furthermore, in untreated controls the serum level of Prl correlated with ODC activity in kidney, liver and pancreas (r between 0.37 and 0.45, $p < 0.05$).

Modulation of kidney, liver and pancreas ODC activity upon FCA. After FCA, these three organ systems showed different reactions. In kidney, ODC activity was decreased on days 1–2; this was similar to the finding with paraffin alone. On days 3–4, kidney ODC activity returned to baseline but remained reduced, particularly during the light phase; this was not observed with paraffin alone.

In liver, ODC activity was further enhanced during the dark phase on days 1–2 and 3–4 (means over 24 h on days 3–4: 55 ± 21 and 79 ± 30 pmol/30 min/mg protein in controls and FCA-treated rats, respectively, $p < 0.05$). These changes were not found with paraffin alone.

In pancreas, ODC diurnal rhythmicity was lost. A marked decrease in pancreas ODC activity was observed on days 3–4 during the dark phase. Similarly, the serum level of insulin was decreased (mean over 24 h on days 3–4: 36 ± 19 μU/ml). These findings resembled those obtained with paraffin alone.

Diurnal rhythm of lung and heart ODC activity. ODC activity in heart and lung (data not shown) was enhanced during the light phase. Compared with controls, paraffin-treated rats showed increased lung ODC activity (mean over 24 h on days 3–4: 30 ± 7 and 45 ± 11 pmol/30 min/mg protein, respectively, $p < 0.05$).

Increased lung and unchanged heart ODC activity upon FCA. After FCA, lung ODC activity was increased to the same extent as after paraffin alone (mean over 24 h on days 3–4: 48 ± 12 pmol/30 min/mg protein, $p < 0.05$ compared with controls). In contrast, heart ODC activity was unaffected.

Diurnal rhythm of lymphoid organ ODC activity. Figure 6 shows the diurnal rhythm of ODC activity in the

primary lymphoid organs. In controls, ODC activity in the thymus and bone marrow, as well as in the secondary lymphoid organs (popliteal lymph nodes and spleen, data not shown), was enhanced during the light phase.

Compared with controls, paraffin-treated rats showed weakly increased thymus ODC activity (mean over 24 h on days 3–4: 86 ± 24 and 119 ± 31 pmol/30 min/mg protein, respectively, $p < 0.05$). Paraffin oil had no effect on the other lymphoid organs.

Increased lymphoid organ ODC activity upon FCA. As expected, after FCA, ODC activity was increased in lymphoid tissues on days 1–2 and 3–4 (fig. 6, table 1). In the thymus, ODC diurnal rhythmicity was lost and activity increased, particularly during the dark phase (mean over 24 h on days 3–4: 264 ± 36 pmol/30 min/mg protein, $p < 0.01$ compared with paraffin-treated rats). In bone marrow, ODC diurnal rhythmicity was preserved (at least on days 3–4), the level being increased, particularly during the light phase (mean over 24 h on days 3–4: 21 ± 6 and 34 ± 7 pmol/30 min/mg protein in controls and FCA-treated rats, respectively, $p < 0.05$). In FCA-treated rats, the serum level of Prl and bone marrow ODC activity were correlated, but only during the dark phase ($r = 0.35$, $p < 0.05$).

On days 1–2 and 3–4, the secondary lymphoid organs (popliteal lymph nodes and spleen) showed a partially disturbed ODC rhythmicity, the activity being significantly increased. Between days 3 and 4, in lymph nodes, the mean over 24 h was 36 ± 6 and 78 ± 16 pmol/30 min/mg protein in controls and FCA-treated rats, respectively ($p < 0.005$); in spleen, the mean over 24 h was 38 ± 7 and 72 ± 9 pmol/30 min/mg, respectively ($p < 0.01$).

Effect of bromocryptine microcapsules. Table 2 shows that pre-treatment with CBLA reduces the basal ODC activity in the pituitary gland, liver and thymus. In controls and FCA-treated rats, Prl was increased during the night (fig. 2). Therefore, we decided to measure the ODC activity in various tissues during the dark phase on days 3–4 (i.e. a period of 12 h). Figure 7 shows the relationship between ODC activity in various tissues and the serum level of Prl during the dark phase in controls and FCA-treated rats. FCA-induced enhancement of ODC activity was inhibited by bromocryptine in the pituitary gland, liver and lymphoid tissues by at least 70%. This was not the case for other tissues tested.

Discussion

In control animals, increased ODC activity is found during the light phase in the pituitary gland, lymphoid tissues, lung, heart and testes. In contrast, enhanced ODC activity occurs during the dark phase in the hypothalamus, liver, adrenal cortex, adrenal medulla, pancreas and kidney. In the pancreas, a similar result has been reported by others [9]. Thus, organs can be grouped differently depending on the diurnal rhythm of ODC activity. Species differences also exist; for example, in contrast to our findings, in mice maximal liver ODC activity occurs during the light phase [8].

The different diurnal rhythms of ODC activity could be due to neuroendocrine fluctuations. Increased serum GH and testosterone are found during the light phase, while Prl, ACTH and CS are elevated during the night. In untreated rats, as expected from previous studies [6, 13, 14], serum Prl correlates with ODC activity in a group of tissues showing peaks during the dark phase (i.e. liver, adrenal cortex, pancreas and kidney).

Apparently, the elevation of serum ACTH during the dark phase is associated with enhanced adrenocortical ODC activity and an increase in serum CS. It is not clear,

Table 2. ODC activity in various tissues after administration of Freund's incomplete (paraffin alone) or complete adjuvant (FCA) in male rats aged 3 months. Effect of pre-treatment with CBLA (1 mg/kg i. m., on day 2). Two rats per groups were killed every 2 h during the dark phase (between 18.00 and 06.00 h) on days 3–4 after FCA (total $n = 42$ per group, mean over 12 h $\pm$ SD). The controls received placebo microcapsules.

	ODC activity (pmol/30 min/mg protein)					
	placebo controls	CBLA alone	paraffin alone	FCA alone	FCA + CBLA	% inhibition[a]
Pituitary gland	35 ± 9	26 ± 4*	37 ± 10	71 ± 12*	27 ± 6*§	97
Thymus	107 ± 25	83 ± 12*	146 ± 29*	232 ± 27*	94 ± 10§	91
Lymph nodes	28 ± 4	24 ± 5	27 ± 6	59 ± 12*	29 ± 4§	84
Spleen	27 ± 4	21 ± 6	24 ± 7	52 ± 8*	26 ± 3§	80
Liver	64 ± 22	43 ± 15*	59 ± 28	89 ± 31*	49 ± 17§	76
Bone marrow	16 ± 3	17 ± 4	18 ± 4	27 ± 5*	20 ± 7	73
Kidney	1029 ± 406	929 ± 309	923 ± 317	1293 ± 490	1098 ± 248	36
Testes	87 ± 34	93 ± 41	53 ± 13*	59 ± 10*	62 ± 9*	-
Pancreas	22 ± 5	20 ± 6	14 ± 7*	10 ± 5*	9 ± 4*	-

[a] $\%\ \text{inhibition} = 100 - \left(\frac{[\text{FCA} + \text{CBLA}] - [\text{CBLA}]}{[\text{FCA}] - [\text{Control}]} \times 100\right)$, the control being the placebo group

*Significant differences with placebo controls (Mann-Whitney U-test, $p < 0.05$)

§Significant differences between FCA alone and FCA + CBLA (Mann-Whitney U-test, $p < 0.05$)

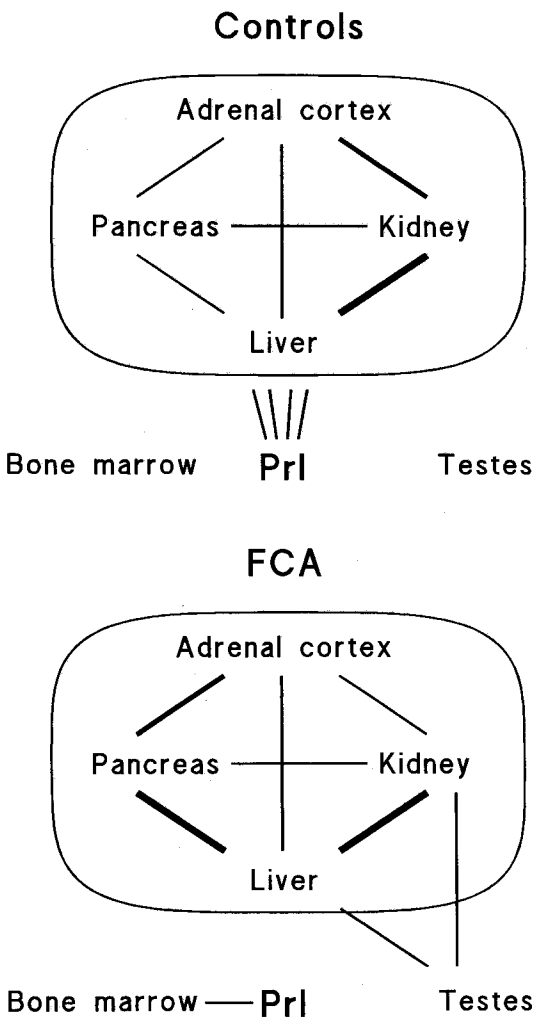

Figure 7. Diagram showing the relationship between ODC activity in various tissues and the serum level of Prl during the dark phase in controls and FCA-treated rats. The lines are proportional to the Spearman's rank correlation coefficients (r between 0.40 and 0.80, $0.05 < p < 0.001$). In untreated rats, serum Prl is associated with ODC activity in a group of four tissues (liver, kidney, pancreas, adrenal cortex). However, in FCA-treated rats, Prl correlated with bone marrow activity only. This suggests different roles for Prl in untreated and FCA-treated rats.

however, whether ODC is induced by the elevation of ACTH [22] or, as suggested by our data, after the release of CS (i.e. reflecting de novo steroidogenesis).

On days 1–2 after FCA, increase of ODC activity occurs in the hypothalamus, pituitary gland, adrenal medulla, adrenal cortex, liver and lymphoid tissues, while ODC activity in kidney is reduced. This is accompanied by an increase in serum CS.

On days 3–4 after FCA, ODC activity remains elevated in the pituitary gland, liver, lung and lymphoid tissues, while the ODC activity in testes, adrenal medulla and pancreas is reduced. In the pituitary gland, this early phase after FCA is associated with increased synthesis of Prl mRNA [5]. In part, this could account for the observed increase of ODC activity since, as expected [23], the peak of activity is inhibited by pre-treatment with CBLA. In studies using bromocryptine mesylate, the timing of Prl inhibition is important in order to obtain an adequate immune suppression. In contrast to the encapsulated form, administration of bromocryptine mesylate in the morning allows an escape of Prl inhibition during the night (data not shown). This could be the reason why CBLA is a more potent inhibitor of AA than the nonencapsulated form. Recently, the importance of suppressing Prl increase during the dark phase also has been demonstrated in mice [21]. Furthermore, on days 3–4 after FCA, adrenal cortex ODC activity is increased during the light phase but decreased during the dark phase. Adrenal cortex ODC activity is reduced during the latency period before development of AA [5].

Increase in ODC activity in the lung and thymus, as well as reduced ODC activity in the testes and pancreas, can be obtained with paraffin alone (Freund's incomplete adjuvant, which does not induce arthritis). This is also the case for reduced testosterone and insulin. Reduced kidney ODC activity observed after either paraffin or FCA could be related to endocrine changes, e.g. decreased testosterone level [24]. The opposite responses of adrenal and kidney ODC resemble the results obtained in rats treated with lithium chloride and/or Prl [25]. Only the early increases in ODC activity in the pituitary gland, liver and lymphoid tissues are specific for FCA (and are not found after injection of paraffin oil).

In untreated control rats and FCA-treated rats, Prl may play different roles. In addition of its well-known effects on reproductive organs and lactation, Prl also influences immune functions. This could occur directly at the level of leukocytes and the cytokine cascade and/or indirectly via structures in lymphoid organs, e.g. the thymus epithelium and its hormones [1, 17]. Footpad immunization causes a rapid, but transient, induction of Prl receptors in the draining lymph nodes [26]. The relation between Prl and the thymus is bidirectional; both forms of Prl receptors are found in rat thymus [27], and thymus hormones can stimulate Prl secretion [28]. In addition, Prl (as GH) can promote erythropoiesis and DNA synthesis in bone marrow precursors [29]. After FCA, there is some evidence that Prl is involved in the enhancement of bone marrow activity (of course, it is not the only factor): the serum level of Prl and bone marrow ODC activity were correlated during the dark phase, and FCA-induced ODC activity in bone marrow, as in other lymphoid tissues, is reduced after pre-treatment with CBLA.

How important are the diurnal time courses described here in the aetiology of an autoimmune response, beyond changes in activity or concentration levels reached at specific phases throughout the light-dark cycle? First,

it was essential to investigate the diurnal rhythms of hormonal changes and ODC activity because the most interesting relationships become obvious during the dark phase. For instance, in controls and FCA-treated rats, Prl is increased during the night. In order to investigate the effect of bromocryptine, we measured ODC activity in various tissues during the dark phase. Secondly, the Prl-dependent process occurring in the early phase upon FCA, i.e. before the onset of arthritis, could influence the ongoing autoimmune process. Macrophage blocking agents, e.g. gold salts, when given i.v. are quite effective injected in just one dose 6 h before FCA [20]. The same is true for pre-treatment with bromocryptine, the encapsulated form (CBLA) of course being more efficient than the free form (CB-154). Sustained Prl suppression could interfere with the efficiency of the cytokine cascade, since synergism of Prl with interferon-γ and interleukin-2 has been described [30, 31].

In male rats under certain circumstances (e.g. a secondary adrenocortical insufficiency) a mild hyperprolactinemia favours the AA reaction [5]. In female mice, injection of Prl makes collagen type II-induced arthritis worse if treatment is performed during the induction stage of the disease, but not later [21]. The situation is very different in established arthritis. In AA, chronic stimulation of the hypothalamic-pituitary-adrenal axis is accompanied by a loss of the diurnal rhythmicity [5, 32]. In the pituitary gland the synthesis of Prl mRNA and GH mRNA are reduced, while the production of pro-opiomelanocortin mRNA (the precursor of ACTH) is increased. It is tempting to speculate that these processes are involved in the decline of the inflammatory reaction (on day 21 and onwards). However, while pre-treatment with bromocryptine microcapsules inhibits AA effectively as a hypophysectomy, the injection of the drug in established arthritis exacerbates the reaction (unpubl. obs.). This adverse effect was also reported in established type-II collagen-induced arthritis [21]. This suggests that Prl (or the dopaminergic pathway) plays different roles in the early and late phases of the autoimmune and inflammatory processes. This must be kept in mind during treatment of patients with bromocryptine.

In summary, the results presented here provide evidence that the increase in ODC activity in the pituitary gland, liver and lymphoid tissues is specific for FCA and is not found after injection of paraffin alone, the so-called Freund's incomplete adjuvant, which does not induce arthritis. A role for Prl in the induction of ODC in liver and lymphoid tissues, but not in other tissues, is suggested by the fact that bromocryptine microcapsules suppress this enhancement. The association between modulation of Prl secretion during the latency period after FCA and the subsequent development of AA is uncertain, but the present work—comparing different organ systems and endocrine parameters—supports this concept.

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