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Interrenal Secretion and Peripheral Production of Cortisone in Eel (*Anguilla anguilla* L.)

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Cortisol/cortisone ratio is about 15 times lower in plasma than in interrenal. The metabolic clearance rate was not significantly different for cortisol and for cortisone. The specific component of blood-binding system had a higher affinity for cortisone. This can account for the small difference observed between their metabolic clearance rates. However, it cannot explain a cortisone plasma storage as great as estimated from cortisol/cortisone ratios.

In vivo (intravenous injection) or *in vitro* (perfusion in closed circuit of isolated head), tritiated cortisol was converted partially to tritiated cortisone and, conversely, tritiated cortisol was produced when tritiated cortisone was used as a tracer. Thus, 11β-hydroxysteroid oxidoreductase is present in eel tissues and, after a few hours, a steady state was attained between cortisol and cortisone.

At level of corticoid target organ as the gill, reactions of oxidoreduction are likely to be involved in the physiological expression of the hormonal action. *In vitro* experiments are in favour of a relation between the gill uptake of cortisol, exchanges of the taken up corticoid and these enzymatic reactions.

Introduction

In eel, like in other teleosts, the predominant circulating corticoid is cortisol but cortisone also exists in plasma, at relatively significant concentration. In peripheral blood, the cortisol/cortisone ratio is about 2 (Lewander et al. 1974, Leloup-Hatey unpublished results). The cortisol/cortisone ratio is similar in the interrenal tissue and in the *in vitro* interrenal secretion; the mean value for the latter is very different from the value found for the plasma ratio (30.1 ± 6.06) (Leloup-Hatey and de Ligniéres 1972). Therefore, it appears that the plasma cortisone cannot be exclusively released by the interrenal gland and it seems interesting to throw light on the problem of its origin in eel.

The plasma ratio of cortisol and cortisone is dependent on the relative rates of their glandular secretion in blood. This ratio can also be modified at tissue level. Metabolic clearance rates of the two compounds may not be comparable owing to the fact that the affinities of plasma proteins for them are different. But it is also possible that one of the compounds is synthesized elsewhere than in interrenal. These two possibilities will be successively considered in order to

explain the apparent cortisone storage in eel plasma. The physiological effect of cortisone at level of corticoid target organs will be discussed in the light of the data presented here.

Materials and Methods

Silver freshwater female eels (*Anguilla anguilla* L.) (body weight : approximatively 200 g) were used some days after their capture in the North of France.

Corticoids were extracted from plasma perfusion medium or gill cells, purified and identified by the usual methods (paper chromatography before and after acetylation, repeated crystallizations to constant specific activity) as previously described (Leloup-Hatey 1976).

Metabolic clearance rates were evaluated by classical methods for the analysis of steroid dynamics, after a single injection or after a continuous injection of tracer according to Leloup-Hatey (1976).

The binding of cortisol and cortisone by the plasma proteins was studied at 4°C by means of equilibrium dialysis method (Plager 1965) in presence of a constant amount of tritiated corticoid and variable amounts of stable corticoid. Experimental points were plotted following the Scatchard method (1949). Association constants and binding capacities of plasma proteins were calculated by the mathematical method of Hart (1965 or by a graphic analysis of experimental results (Rosenthal 1967).

Isolated heads were perfused through the ventral aorta in closed circuit by means of special pump under variable pressure according to Kirsch and Colin (unpublished method). The tracer was added to the perfusion medium (20 p. 100 eel plasma-80 p. 100 Krebs-Ringer buffer, *p*H 7.2). At the end of perfusion, the isolated head preparation was rinsed for 5 min by the Krebs-Ringer buffer. The gill arches were then isolated and the cells were gently removed from branchial lamellae for a chromatographic study of radioactivity.

Results

(i) *Metabolic clearance rates of cortisol and cortisone*

In eel plasma, a protein system with two components was responsible for the corticoid binding (table 1). The association constant K_1 of the component of high affinity and low capacity was 3 to 4 times higher for cortisone than for cortisol but it was much lower than those of mammalian transcortins. The association constant K_2 of the low affinity and large capacity component was not different for cortisol and for cortisone and it was near those of mammalian serum albumins. Lastly, binding protein sites were common for the two corticoids because one could compete with the other in the dialysis system. It was only necessary to add more

Table 1 *Binding of two corticosteroids to eel plasma proteins: Association constants at 4°C and binding capacities*

	Component of high affinity and low capacity		Component of low affinity and high capacity	
	Association constant	Binding capacity	Association constant	Binding capacity
	[10^6 1. M^{-1}]	[μ Mol]	[10^4 1. M^{-1}]	[μ Mol]
Cortisol	9.97 (8)	0.13 (8)	6.7 (8)	8.7 (8)
Cortisone	39.3 (3)	0.14 (3)	7.2 (3)	8.8 (3)

Number of determinations are given in parentheses

cortisol than cortisone to get the same competitive effect.

Consequently, the metabolic clearance rate of cortisone was lower than that of cortisol; but the difference was not statistically significant (table 2). The values obtained by the two methods (single or continuous injection of tracer) were not significantly different. Some variations in experimental conditions can be the cause of small observed differences since, in eel, the metabolic clearance rate of corticoids varies with season (Leloup-Hatey & Hardy 1976a) and, above all, with the water temperature (Leloup-Hatey & Hardy 1976b).

Table 2 *Plasma metabolic clearance rates of corticosteroids in freshwater eels (ml plasma/day/kg body weight)*

	Single injection of tracer	Continuous injection of tracer	Comparison between the two methods
Temperature Season	T° = 10–12° Winter	T° = 9–10° Spring	
1.2 (^{3}H) — Cortisol	1680 ± 66.7 (5)	1345.7 ± 102.5 (3)	0.1>P>0.05
1.2 (^{3}H) — Cortisone	1446 ± 187.8 (7)	1189.3 ± 168.75 (6)	0.4>P>0.3
Comparison of clearance rates of the two corticoids	0.3>P>0.2	0.6>P>0.5	

The metabolic clearance rate is evaluated :

After a single injection of tracer, by the numerical integration of hormone disappearance curves as described by *Fortier and Normand* (1970).

After continuous injection of tracer by the ratio: $\frac{\text{Injected dose by day in dpm}}{\text{dpm of [Cortisol + Cortisone]/ml plasma}}$

(ii) *Tissue synthesis of cortisone*

A. *In vivo experiments*

After intravenous injection of tritiated cortisol, tritiated cortisone quickly appeared in plasma and its level increased as a function of time, until it reached a maximal value (figure 1). A similar percentage of tritiated cortisone was found in plasma after a long enough infusion of tritiated cortisol resulting in a constant specific activity of the plasma cortisol. The plasma equilibrium is reached with two parts of tritiated cortisol against one part of tritiated cortisone. This observation suggests that the transformation of cortisol to cortisone is a reversible phenomenon and this hypothesis is confirmed since after intravenous injection of tritiated cortisone, tritiated cortisol was found in plasma.

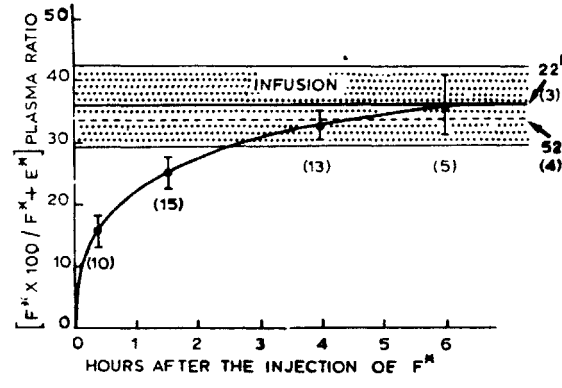

Figure 1 Kinetics of 1,2 (^{3}H)-cortisone (E*) in the eel plasma after intravenous injection of 1,2 (^{3}H)-cortisol (F*)

B. *In vitro experiments* (in collaboration with D. Colin)

After perfusion of isolated head in closed circuit, the two corticoids were always

present in the perfusion medium, whatever tracer was in this medium : tritiated cortisol or tritiated cortisone (figure 2). Concentration of synthesized corticoid regularly increased as a function of time. A positive significant correlation related the percentage of synthesized corticoid to the duration of tracer perfusion. A covariance analysis of data did not show any significant difference between the slopes of these curves.

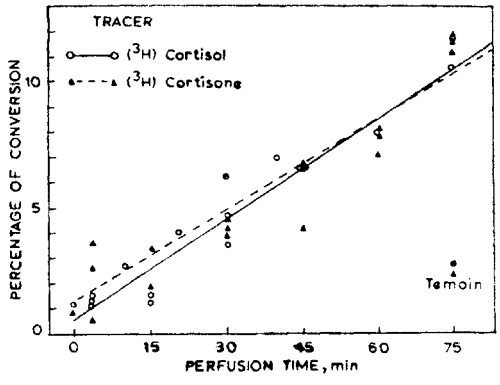

Figure 2 Kinetics of *in vitro* conversion of 1,2(^{3}H)-cortisone to 1,2 (^{3}H)-cortisol. (*J. Leloup-Hatey and D. Colin, unpublished results*)

A positive significant correlation was shown between the percentage of corticoid conversion (y) and the perfusion time (x),

Cortisol : $y=0.135\ x + 0.398$ ($r=+0.969$)
Cortisone : $y=0.124\ x + 1.150$ ($r=+0.936$)

(iii) *Gill cortisone uptake* (in collaboration with D. Colin)

The gill cells, removed from branchial lamellae after a continuous perfusion of isolated head with medium containing either tritiated cortisol or tritiated cortisone, retained a significant quantity of tritium. This radioactivity was really taken up by cells, since it was still found after a 5 min rinsing perfusion with Krebs-Ringer buffer without tracer (table 3). Most of the radioactivity was washed away from the perfused head during the first 2 min of rinsing (figure 3). A chromatographic study of gill radioactivity showed that the gill cell only took up

Table 3 *Perfusion of isolated head of eel* (*J. Leloup-Hatey and D. Colin, unpublished results*)

Perfusion Medium — Krebs Ringer Buffer *p*H 7.4..... 8 ml
Eel plasma..... 2 ml
Tracer..... 1 µCi

Tracer	Total Tritium (dpm)			Cortisol (p. 100 of total tritium)			Cortisone (p. 100 of total tritium)		
	Medium Perfusion (ml)*	Gills (g)†	Rinsing Buffer	Medium Perfusion	Gills	Rinsing Buffer	Medium Perfusion	Gills	Rinsing Buffer
	46 815	86 996	12 406	82.45	99.9	74.7	8.25	Tr.	11.1
Cortisol	97 763	53 170	8 532	79.9	91.8	80.5	10.1	Tr.	12.65
	96 335	45 202	7 273	81.5	97.45	57.5	9.7	Tr.	7.0
		Mean :		81.3	96.4	70.9	9.35	Tr.	10.25
	56 307	27 001	7 812	9.6	97.2	14.05	72.4	Tr.	31.05
Cortisone	61 970	32 208	11 126	10.05	99.5	10.3	74.25	Tr.	38.7
	73 947	37 815	8 845	9.5	99.0	3.8	74.9	Tr.	34.9
		Mean :		9.7	98.6	9.4	73.85	Tr.	34.9

*Medium after 75 min of continuous perfusion

†Gills after 5 min of rinsing by buffer without eel plasma

cortisol, even when tritiated cortisone was the perfused tracer (table 3).

Discussion

In Salmon, cortisone is found in plasma (Idler et al. 1959, in *Oncorhynchus nerka*; Hane & Robertson 1959, in *Oncorhynchus tschawytscha*; Leloup-Hatey 1964, in *Salmo salar*) but its concentration is relatively higher than in the eel plasma. The metabolic clearance rate of cortisone is significantly lower than the metabolic clearance rate of cortisol (Donaldson & Fagerlund 1968). Strong binding of cortisone by plasma proteins may explain this difference; in fact, the affinity of plasma proteins for cortisone is about 10 times that for cortisol (Freeman & Idler 1971). Also, in salmon, there is a

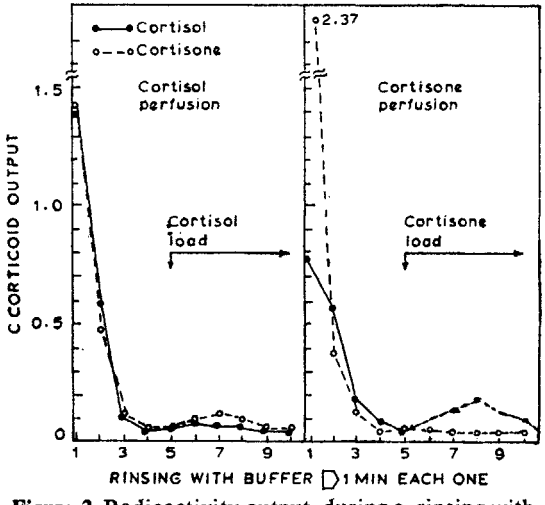

Figure 3 Radioactivity output during a rinsing with Krebs-Ringer buffer of the isolated head perfused for 75 min with a tritiated corticoid

(J. Leloup-Hatey and D Colin, unpublished results)

Tritiated corticoid output was measured in the rinsing medium. The radioactivity of fractions collected each min was related to the radioactivity of the 75 min perfusion medium.

plasma storage of the cortisone released by the interrenal gland principally imputable to this property of binding plasma proteins.

It may be asked whether the relatively high concentration of cortisone in eel plasma can be explained in the same way? In this species, the metabolic clearance rate of cortisone is a little lower than that of cortisol. As in salmon, the affinity of plasma proteins is also higher for cortisone but it is only 3 to 4 times that for cortisol. This suggests that the metabolic clearance rates of the two corticoids are not significantly different in eel. Yet, it appears that the difference of these rates is not large enough to determine, as in salmon, a notable storage in plasma of the cortisone released by the interrenal gland.

In vivo experiments prove the cortisone synthesis in eel tissues: the circulating cortisol is oxidized in cortisone. In course of time, a steady state is reached in the plasma between the hormone (2 parts of cortisol) and its metabolite (1 part of cortisone). Such an equilibrium state involves a simultaneous reduction of cortisone to cortisol. This reduction is also shown by *in vivo* experiments, after injection of tritiated cortisone. It has been very clearly demonstrated by *in vitro* experiments on isolated head. So, in eel, some plasma cortisone is released by the interrenal gland but it appears that it is principally synthesized in other tissues. Such a tissue synthesis probably takes place in salmon too. In *Oncorhynchus nerka*(Donaldson & Fagerlund 1972), a study of tissue cortisol/cortisone ratio has indicated a rapid conversion of cortisol to cortisone in some of the tissues (spleen, heart, gut, gills), and also of cortisone to cortisol in other tissues (liver). However, as these conversions have probably a small magnitude in salmon, they were not taken into consideration to explain the high concentration of cortisone in the plasma in this species.

In vitro kinetics of the processes of cortisol oxidation and of cortisone reduction are not different. Consequently, the level of the "cortisol-cortisone" equilibrium in plasma

may be dependent on a single type of enzymatic tissue activity : an 11β-hydroxysteroid oxidoreductase, like the one identified in mammals and in birds. Nevertheless, in steady state, the plasma level of "reduced hormonal metabolite" is higher in fishes (cortisone) than in mammals (cortisone or 11-dehydrocorticosterone) and in birds (11-dehydrocorticosterone). The relatively high concentration of cortisone in fish plasma could be due to higher affinity of plasma proteins for cortisone than for cortisol. In normal conditions, this higher affinity for cortisone seems to favour the exchange of the cortisone produced in cell against plasma cortisol, which enters the cell and is taken up principally in corticoid target cell.

In view of the results of *in vitro* experiments, it is interesting to discuss the physiological importance of the described reactions of 11β-hydroxysteroid oxidoreduction. The cortisol is selectively taken up by gill cells, a corticoid target tissue in teleosts(Maetz 1969). This result confirms the *in vivo* experiments of Goodman and Butler (1972) which showed a very small cortisol accumulation in eel gill. The 11β-hydroxysteroid oxidoreductase activity is probably in relation with the exchanges of hormone retained in the gill. This enzymatic activity, localized in gill cells, or in some other cephalic tissues, would be involved in the *hormonal contribution to the target tissue : in vitro*, the perfused cortisone is reduced to cortisol which is taken up by gill cells. It is also probably responsible for the *renewal of cortisol* taken up by gill cells. Some preliminary results suggest that a release of tritiated cortisol taken up by gill cells of isolated head is observed after a cortisone overload of the preparation.

Reactions of oxidoreductions of this type are probably a general phenomenon in corticoid target tissues. They would play a role in the physiological expression of hormonal action either directly at the nuclear level, or by allowing an increase in energy balance within the target cell.

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