Inactivation of Vasotocin by Hen's Tissues in vitro

Neurohypophysial hormones are rapidly degraded by tissue homogenates, mostly on proteolytic or reductive pathways[1-3]. Vasotocin (8-arginine oxytocin) and mesotocin (8-isoleucine oxytocin) are present in the neurohypophysis of the hen[4,5]. Vasotocin is the most potent oxytocic substance in this species and is probably concerned with oviposition[4,6-8]. Its half-life in the hen's blood is rather long and amounts approximately 20 min[9]. It has been anticipated that, as in mammals, the hormone in the hen is partly excreted and partly inactivated by the tissues[9].

In previous studies from this laboratory, it has been found that oxytocin is inactivated by hen's plasma[10]. Since knowledge on the peripheral degradation of vasotocin in birds is still lacking, the present work has been undertaken to examine the inactivation of this hormone by homogenates of liver, kidney and oviduct of the hen.

Experimental. The liver, kidney and oviduct of laying-hen (no egg in utero) were homogenized at 0 °C in solution containing 0.01 *M* Tris, 0.0015 *M* $MgCl_2$ and 0.01 *M* KCl (pH 7.0). The tissue homogenates were incubated at 37 °C for 5, 10, 15 min (liver, kidney, uterus) or for 15, 30, 60 min (magnum, isthmus, vagina) with vasotocin (Sandoz Ltd, Basle). 1 ml of incubation mixture contained: 0.05 µg of vasotocin; 0.1 ml of 20% w/v tissue homogenate; 0.2 ml of 0.1 *M* sodium-phosphate buffer (pH 7.0) and 0.6 ml of water. The tissue enzymes were inactivated by heating for 3 min in boiling water. The residual hormonal activity was tested in the 4-points system according to BURN et al.[11] on the laying-hen's uterine strips suspended in medium of MUNSICK et al.[12] (with magnesium ions) at a temperature of 42 °C and a flow rate of 12 ml/min. Protein concentration in homogenates were estimated by Kjeldahl method.

Results and discussion. It has been found that the hen's tissue homogenates (liver, kidney and parts of oviduct: magnum, isthmus, uterus and vagina) are able to inactivate vasotocin. The liver and uterus homogenates showed the highest inactivating activity; kidney about half that of the liver. Relatively little activity was found in other tissues.

The rate of inactivation of vasotocin expressed in the half-life of the hormone or in the first order constant of inactivation are given in Table I. The shortest half-life (4.75 min) was obtained for the liver and the longest (240 min) for the magnum. In the case of uterus a biphasic course of the inactivation rate has been noted. After the first 5 min of a rapid hormone inactivation (amounted to 50% of the previous activity), a phase of a very slow inactivation was followed.

Relative potency for inactivation of various parts of the hen's oviduct increased from the ovary toward the uterus (Figure). Similarly increased sensitivity of the smooth muscles of the oviduct to vasotocin[13].

In the Figure, the amounts of inactivated vasotocin by tissue homogenates were compared with a relative sensitivity of different parts of the hen's oviduct to this

Table I. Inactivation of vasotocin in homogenates of various tissues

Tissue[a]	Half-life (min)	Hydrolytic rate constant[b] ($min^{-1} \times 10^{-2}$)
Liver	4.75	14.50
Kidney	9.00	7.70
Oviduct		
Uterus	5.25	13.20
Vagina	37.50	1.84
Isthmus	75.00	0.92
Magnum	240.00	0.28

Mean values of 5 experiments. [a] Homogenate concentration 20% w/v. [b] First order constant of inactivation.

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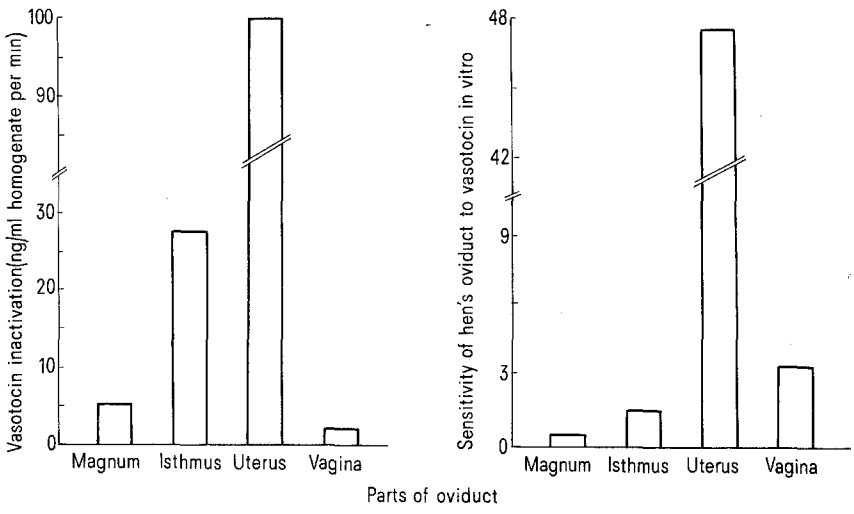
Table II. Relationship between the amount of inactivated vasotocin and the total protein content in different tissues homogenates

Tissue	Protein concentration mg/ml (mean ± S.E.)	Vasotocin inactivation	
		ng/mg protein per min (mean ± S.E.)	µU/mg protein per min (mean ± S.E.)
Liver	38.84 ± 12.9	0.142 ± 0.016	36.20 ± 4.35
Kidney	28.72 ± 29.8	0.113 ± 0.033	30.56 ± 8.40
Oviduct			
Magnum	40.92 ± 29.0	0.008 ± 0.0009	2.06 ± 0.12
Isthmus	66.5 ± 2.2	0.026 ± 0.003	6.64 ± 0.90
Uterus	109.4 ± 13.9	0.501 ± 0.094	125.49 ± 24.01
Vagina	80.2 ± 3.3	0.047 ± 0.007	11.70 ± 1.93

Mean values of 5 experiments.



Comparison between the degree of vasotocin inactivation by hen's oviduct homogenates and sensitivity of the oviduct to vasotocin. Sensitivity of uterus taken as 100 (calculated by RZĄSA[13]).

hormone. Of the 4 parts of oviduct examined, the uterus showed the greatest sensitivity to vasotocin and simultaneously possessed the highest ability to inactivate this hormone.

The total protein content in tissue homogenates ranged from 6.65 (isthmus) to 40.92 mg/ml (magnum) (Table II). No relationship between the protein concentration and the vasotocin-inactivating activity was observed. Tissue inactivating ability expressed in ng of inactivated vasotocin/mg of protein per 1 min was 3 times greater in the case of uterus than in the liver. However, the rate of vasotocin inactivation by these tissues expressed in half-life was similar (Table I).

Ability of the hen's liver and kidney tissues to inactivate neurohypophysial hormones resembles that found in mammals. On the other hand, the striking phenomenon observed was the high vasotocin-inactivating activity of uterine portion of hen's oviduct. In mammals, uterus inactivates oxytocin approximately 10 times less than the liver and kidney[14,15]. It is probable that the high ability of hen's uterus to inactivate vasotocin is associated with a great sensitivity to vasotocin, as found in vitro and in vivo studies[4,8,13]. This point of view might be in general agreement with studies on the other hormones which shows that the tissues which are metabolically sensitive to the hormone possess simultaneously a high degree of inactivating activity to this hormone[16].

As found in our previous work, the inactivation of oxytocin in hen's, by contrast to mammals, occurs also in serum, irrespectively of the sex[10]. The enzyme of L-cystine aminopeptidase activity identified in hen's serum could be responsible for this inactivation[17]. In relation to vasotocin, HASAN and HELLER[9] did not observe an ability of hen's plasma to inactivate this hormone.

The results reported give no information about the mechanisms by which the biological activity of the vasotocin dissappeared from the circulation of the hen. It may be, that in the process of its inactivation both the aminopeptidases and disulfide reductases take part. It cannot be excluded also that bird tissues contain an enzyme of the endopeptidase type, similar to that found in rat[18] and kidneys[2] and possessing ability to release of glycinamide from the molecule of neurohormone[19].

Zusammenfassung. In vitro wurde die Inaktivation von Vasotocin in einigen Gewebehomogenaten (Leber, Niere, Eileitersegmenten, Magnum, Isthmus Uterus, Vagina) festgestellt. Die grösste Inaktivation wurde bei den Homogenaten von Leber und Uterus gefunden.

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Effect of *Bordetella pertussis* Vaccine on Plasma Corticosterone Level and on ACTH-Induced Corticosterone Secretion in Rats

Both adrenalectomy and *Bordetella pertussis* vaccine (BPV) increase the sensitivity of mice and rats to anaphylactic shock, shock-mediators, various toxic and stress effects[1–6]. It was earlier suggested by KIND[7] that BPV exerts its effect by injuring the adrenal cortex. However, this supposition has not been confirmed in mice[2,8]. Our recent investigations regarding the histamine metabolism of BPV-treated rats again raised the possibility that reversible adrenal insufficiency develops following BPV sensitization[9]. The purpose of the present study was to examine whether BPV influences plasma corticosterone level and ACTH-induced corticostrone secretion in rats.

Materials and methods. Female Wistar rats (150–200 g) were maintained on standard diet and drinking water ad