Fluoreszenzmikroskopische Untersuchungen am Hypothalamus männlicher Ratten nach Behandlung mit einer antimaskulin wirkenden Substanz

NEUMANN et al.[1-4] konnten feststellen, dass Antiandrogene, wenn sie in den ersten 3 Lebenswochen appliziert werden, männlichen Ratten ein irreversibles weibliches Sexualverhalten aufprägen. Diese Befunde liessen vermuten, dass die hypothalamischen Sexualzentren ein wichtiges Erfolgsorgan für Antiandrogene sind. Zu den Sexualzentren gehören die kleinzelligen Kerngebiete im vorderen Hypothalamus und vor allem der Nucleus arcuatus[5-7]. Da bisher nichts Näheres über den Angriff der Antiandrogene am Hypothalamus bekannt ist, versuchten wir mit Cyproteron-Azetat (CA), einem starken Antiandrogen, in die vom Dienzephalon gesteuerten Regelmechanismen der Sexualfunktion einzugreifen.

Wir haben männlichen Ratten, beginnend am 1. Lebenstag, täglich 1 mg CA s.c. injiziert und uns zum Nachweis der Wirkung des CA auf den Hypothalamus der fluoreszenzmikroskopischen Darstellung von Katecholaminen am Kryostatschnitt[8] bedient. Es waren Verschiebungen im Katecholamingehalt des Zwischenhirns zu erwarten, da bekannt ist, dass bei weiblichen Ratten zyklusabhängige Schwankungen biogener Amine im Hypothalamus auftreten[9].

Durch die Behandlung mit CA wird das Auftreten optimaler Fluoreszenz während der postnatalen Entwicklung um etwa 10 Tage vorverlegt – vom 35. Lebenstag beim Normaltier auf den 25. beim behandelten Tier. Ferner kommt es zu einer bemerkenswerten Fluoreszenzzunahme im Bereich des vorderen Hypothalamus sowie der Nuclei peri-, paraventricularis und supraopticus. Die Fluoreszenzvermehrung erfolgt in erster Linie in den Nervenfasern. In den kleinzelligen hypothalamischen Kernen tritt sie auch in einzelnen Ganglienzellen auf, während in den grosszelligen Arealen die Perikarya stets fluoreszenzfrei sind. Nach CA-Behandlung erscheinen an der Oberfläche der Nervenzellen in den magnozellulären Gebieten vermehrt punktförmig endende katecholaminreiche Nervenfasern. Die Region des Nucleus paraventricularis ist katecholaminreicher als die des Nucleus supraopticus. – Die beim Normaltier stark fluoreszierende Zona externa der Eminentia mediana leuchtet nach Antiandrogenbehandlung noch stärker. Ausserdem ist die fluoreszierende Zone gegenüber der Norm stark verbreitert, und es treten zahlreiche katecholaminhaltige Nervenfasern mit den Spezialarterien der Zona interna in Verbindung. – Die gleichen Befunde konnten wir bei männlichen Ratten nach Kastration am 1. Lebenstag erheben. Diese Beobachtung deckt sich mit dem biochemischen Nachweis eines vermehrten Katecholaminvorkommens im vorderen Hypothalamus nach Kastration[10] und der elektronenoptisch nachweisbaren Zunahme monaminhaltiger Granula[11]. Ferner bestätigen unsere Befunde die von NEUMANN et al.[3] getroffene Feststellung, dass die Behandlung mit CA in den ersten Lebenswochen einer Kastration am ersten Lebenstag gleicht[12].

Aus unseren Versuchen ist zu folgern, dass die hypothalamischen Sexualzentren über den durch das Antiandrogen gestörten Feed-back-Mechanismus in einen Zustand der Überfunktion geraten, die mit einer vermehrten Katecholaminproduktion verbunden ist. Hiermit geht wahrscheinlich eine vermehrte Produktion von Gonadotropin-«releasing-factors» einher, die möglicherweise nach CA-Behandlung in der gleichen Form wie nach Kastration[3] abläuft.[12]

Summary. In new-born male rats daily injections of a strong antiandrogenic substance led to an increase of the catecholamine content in the parvicellular perikarya as well as in nerve fibres leading to the paraventricular and supraoptic nuclei. The same effect was observed in male rats castrated immediately after birth. A possible relationship between gonadotropic releasing factors and the catecholamin content of the hypothalamic nuclei is discussed.

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Inhibition of Adrenal Cortical Hormones by an Extract of Lymphoid Tissue

ACTH and adrenal corticosteroids have a dramatic, favorable effect in allergic states and in 'collagen' diseases. These hormones also cause a decrease and dissolution of lymphocytes with release of lymphocytic cellular constituents[1,2]. These observations suggest that the favorable effect of ACTH and corticosteroids in hypersensitivity and in the diseases of the connective tissue might be mediated by some components of the lymphoid tissue. If this be true, it might be possible to isolate a substance or substances from the lymphoid tissue with therapeutic properties of corticosteroids and, hopefully, without the undesirable side effects of these hormones. This would also imply that lymphoid tissue represents a target organ in a lypothalamus-anterior pituitary-cortical adrenal-lymphopoietic tissue system. In such a system

[1] T. F. DOUGHERTY, in *Kinetics of Cellular Proliferation* (Grune-Stratton, New York 1959), p. 264.
[2] J. H. HUMPHREY and R. G. WHITE, in *Immunology*, 2nd edn (F. A. Davis Co., Philadelphia 1964), p. 373.

the cellular components of the lymphoid tissue would most likely exercise a feedback effect on the anterior pituitary-cortical adrenal endocrine axis.

In the past, we have presented data which suggested that lymphoid tissue could interfere with process of immunization and hypersensitization[3,4].

Assuming a feedback effect of the lymphoid tissue on the anterior pituitary or the cortical adrenal gland, we studied the effect of an aqueous bovine extract of lymphoid tissue on plasma cortisol and corticosterone (B) and the urinary 17-hydroxycorticosteroids and 17-ketosteroids in 33 rabbits and 10 dogs. We also studied the effect of lymph node extract in plasma 11-desoxycortisol in animals under treatment with methopyrapone, in order to assess the effect of lymphoid tissue on the anterior pituitary gland versus cortical adrenals.

Methods. Beef mesenteric lymph nodes were removed within 10 min after the animals have been killed and were preserved in dry ice until the time of extraction. The remaindure of the procedure was carried out in a cold room at a temperature of 0–5 °C. Extraneous material, fibrous tissue and fat, were carefully dissected from the nodes. The lymph nodes were then passed through a meat grinder. The ground material was extracted with 5 volumes of 0.15 *M* NaCl by using gentle agitation. This suspension was homogenized in an ordinary Waring Blender at 10,000 rpm for 5 min. The pH of the suspension, which varied between 5 and 6.7, was adjusted to 7.4 by adding an appropriate volume of normal $NaHCO_2$. The extract was then stored in the cold room for 1 h. Then it was filtered through a series of filters in the following order: cheese cloths, Whatman unwashed Filter Paper, No. 1 and No. 3, and Seitz bacterial filter. The lymph node extract was sterile by bacterial culture methods and could be used parenterally. A sterile bovine kidney extract was made by the same method and was substituted for the lymph node extract in the control studies.

Eighteen rabbits, averaging 2.5 kg, were used to study the effect of lymph node extract on the urinary 17-hydroxycorticoids. In 8 of these animals 17-ketosteroids were also determined. The urinary 17-hydroxycorticoids and 17-ketosteroids were determined in a 72-h urine specimen collected before and in a 72-h urine specimen collected while animals were under treatment with lymph node extract. The treatment consisted of 3 ml extract given i.m. at 4 h intervals. These hormones were determined by the PORTER and SIEBER method[5].

The effect of the lymph node extract on plasma cortisol and corticosterone (B) was studied on 15 rabbits averaging 2.5 kg. Plasma cortisol and corticosterone (B) were determined before and at the end of a 3 day treatment with lymph node extract[6]. The treatment consisted of 3 ml aqueous lymph node extract given i.m. at 4 h intervals.

Plasma cortisol was also studied in 5 dogs treated with lymph node extract, and in another 5 dogs used as controls, in which kidney extract was substituted for lymph node extract. The dogs weighed between 10 and 12 kg. The treatment consisted of 10 ml aqueous solution of lymph node extract or kidney extract, given i.m. every 4 h for 3 days. Plasma cortisol was determined at 08.00 on day one, before treatment with lymph node or kidney extract, and at 12.00 and 18.00 on the same day, treatment was started. It was again tested at 8.00 on the second and third day while the animals were under treatment with lymph node extract or kidney extract.

Methopyrapone inhibits 11-β-hydroxylase in the corticoadrenals. As a result of this inhibition, plasma cortisol decreases. Decreased plasma cortisol level diminishes the inhibitory effect of this hormone on ACTH; consequently plasma ACTH level increases causing an overstimulation of cortical adrenals. This leads to overproduction of adrenal cortical hormones in the form of 17-ketogenic steroids, 17-ketosteroids, 17-hydroxycorticosteroids and 11-deoxycorticosteroids. The plasma 11-deoxycorticosteroid determination[7–9] provides the best index of the effect of the methopyrapone on 11-β-hydroxylase. The effect of methopyrapone on plasma 11-deoxycorticoids with and without the interference of lymph node extract indicate the site of an eventual inhibitory effect of lymphoid tissue on the anterior pituitary-cortical adrenal endocrine system. This effect was studied in 5 dogs, averaging 10–12 kg; 5 other dogs were used as controls, in which kidney extract was substituted for lymph node extract. Methopyrapone was given orally, 500 mg every 4 h for 3 days. Lymph node extract and kidney extract were started 24 h later and were given i.m., 10 ml at 4 h intervals for 48 h. Plasma cortisol was determined in blood specimens taken before treatment with methopyrapone, 12 and 24 h after this treatment was started, and every 6 h during the first day of treatment with both methopyrapone and lymph node extract or kidney extract. A last blood specimen was taken 24 h after the beginning of this treatment.

Results. There was a constant and significant decrease in the plasma cortisol and corticosterone (B, Table I) levels in 14/15 rabbits, and there was a marked decrease in the urinary 17-hydroxycorticoids in all 18 rabbits and the 17-ketosteroids in 6/8 animals (Table II) after a 3

Table I. Effects of a 3-day treatment period with lymph node extract on plasma cortisol and corticosterone (B) in mcg/100 ml

Rabbit	Cortisol (mcg)		Corticosterone (mcg)	
	Before	After	Before	After
1	2.02	less 0.12	76.0	18.3
2	0.88	less 0.12	102.0	22.5
3	1.15	less 0.12	78.0	58.2
4	1.83	0.32	73.0	30.0
5	2.11	2.06	89.0	86.0
6	0.96	less 0.12	112.0	26.0
7	1.76	0.32	92.0	30.0
8	2.06	less 0.12	86.0	22.0
9	2.15	less 0.12	104.0	48.0
10	1.88	less 0.12	76.0	20.0
11	1.54	less 0.12	98.0	52.5
12	2.00	0.64	120.0	64.5
13	1.68	less 0.12	112.0	24.0
14	2.25	less 0.12	92.0	42.0
15	1.95	0.30	116.0	48.0

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Table II. Effects of a 3-day treatment period with lymph node extract on urinary 17-hydroxycorticoid and 17-ketosteroid excretion in mg/72 h

Rabbit	17-Hydroxycorticoid (mg)		17-Ketosteroid (mg)	
	Before	After	Before	After
1	1.98	0.71	0.90	0.10
2	0.56	0.10	0.90	n.s.
3	0.71	0.38	0.50	0.30
4	1.47	0.86	0.70	0.30
5	0.96	0.18	0.40	0.10
6	5.04	0.00	1.20	2.20
7	1.38	0.00	0.50	0.30
8	1.65	0.00	0.90	0.80
9	0.26	0.00		
10	0.15	0.00		
11	0.90	0.30		
12	0.30	0.06		
13	0.40	0.20		
14	0.70	0.40		
15	0.50	0.09		
16	2.10	0.00		
17	1.10	0.00		
18	0.90	0.40		

Table IV. Plasma compound S in dogs treated with methopyrapone and lymph node extract or kidney extract (mcg/100 ml)

Treatment	Day	(time)	Dogs 1	2	3	4	5
Methopyrapone	1	08.00	11.7	14.6	19.0	16.0	6.9
		20.00	22.8	14.4	12.0	24.0	9.8
Methopylone and lymph node extract	2	08.00	32.5	16.5	24.7	25.0	33.0
		14.00	5.8	10.2	2.9	20.0	4.6
		20.00	11.6	10.7	4.5	18.0	34.5
	3	08.00	11.2	12.6	4.6	11.0	34.5
Methopyrapone	1	08.00	3.1	4.9	12.6	9.8	10.8
		20.00	11.9	12.7	16.5	3.4	28.1
Methopyrapone and kidney extract	2	08.00	24.4	13.4	21.3	14.2	23.0
		14.00	18.4	11.2	19.7	14.7	30.0
		20.00	17.2	12.1	15.8	12.9	29.0
	3	08.00	18.1	10.6	16.2	12.9	28.0

day treatment with lymph node extract. Plasma cortisol level fell in 4/5 dogs treated with lymph node extract. With one exception, these findings were not seen in the control animals in which kidney extract was substituted for lymph node extract (Table III). In the 2 groups of dogs treated with methopyrapone and lymph node extract or kidney extract, there was a constant rise in the plasma tetrahydro-11-deoxy-cortisol (Compound S) as expected, during treatments with methopyrapone. When lymph node extract was added to the methopyrapone treatment, plasma tetrahydro-11-deoxy-cortisol fell in 4/5 dogs below the levels seen during treatment with methopyrapone alone. These results could not be duplicated in the animals treated with methopyrapone and kidney extract, except for 1 animal (Table IV).

Discussion. The results of these experiments suggest a feedback effect of lymphoid tissue on the anterior pituitary cortical adrenal endocrine axis. Whether the inhibitory effect of the lymphoid tissue affects the pituitary or the adrenals is not absolutely clear. The experiments in which animals were treated with lymph node extract while under methopyrapone stimulation, seem to indicate that the site of lymphoid tissue inhibition is probably in the pituitary.

Data obtained from these experiments also indicate that lymphoid tissue could represent a target gland in a hypothalamus-anterior pituitary-cortical adrenals-lymphopoietic system; and it might be possible that some of the therapeutic effects attributed to ACTH and corticosteroids are, in fact, due to some of the cellular components of the lymphoid tissue. It is therefore conceivable that one might be able to isolate a substance or substances of this tissue with therapeutic properties and without the undesirable effects of the corticosteroids[10,11].

Table III. Plasma cortisol in dogs treated with lymph node extract and kidney extract (mcg/100 ml)

Extract	Day	(time)	Dogs 1	2	3	4	5
Lymph node extract	1	08.00	26.9	22.2	5.0	2.2	6.0
		14.00	16.1	16.6	5.6	1.1	1.6
		20.00	14.4	3.0	4.5	0.6	7.1
	2	08.00	7.2	7.7	1.0	1.0	13.8
	3	08.00	4.4	8.3	2.8	1.2	13.3
Kidney extract	1	08.00	6.1	17.5	22.2	10.6	2.1
		14.00	13.3	14.0	20.0	13.9	2.3
		20.00	9.5	8.0	7.8	8.8	1.8
	2	08.00	7.2	12.2	5.6	10.1	2.8
	3	08.00	7.2	19.4	11.2	8.8	2.5

Résumé. Les résultats obtenus indiqueraient une relation étroite entre le tissu lymphatique et les glandes endocrines, l'hypophyse et les corticosurrénales. Les auteurs suggèrent la possibilité d'un système endocrinaire, comprenant l'hypothalamus, l'hypophyse, les surrénales et le tissu lymphatique.

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