A Study of the Antigonadotrophic Action of Synthetic Arginine Vasotocin

In 1963, MILCU et al.[1] reported the occurrence of a polypeptide with pressor and oxytocic activities in the bovine pineal body. On the basis of pharmacological and paper chromatographical data of a partially purified product they concluded this polypeptide to be identical or related to vasotocin. We investigated acetone-dried powders of bovine and sheep pineal bodies by extraction and purification procedures which are well established for neurohypophyseal peptides, but we have not been able to confirm this work of MILCU et al. (see EBELS et al.[2]). Recently PAVEL and PETRESCU[3] published experimental data which, according to these authors, demonstrate that the stimulatory action of pregnant mare serum (PMS) on mice uteri and ovaries can be inhibited by a partially purified pineal body product and by synthetic arginine vasotocin. They conclude from their data, that the inhibitory principle in bovine pineal body is identical with arginine vasotocin. As we are working on antigonadotrophic sheep pineal activity[4] and as our attempt to isolate arginine vasotocin from sheep and bovine pineal bodies[2] was not successful, we have studied the inhibition of gonadotrophic activity by synthetic arginine vasotocin. These results are compared with the data we obtained previously with sheep pineal powder[4] and sheep pineal extracts[5].

Materials and methods. The synthetic arginine vasotocin was a gift from the Squibb Institute for Medical Research, New Brunswick, N.J., which we received through the kindness of Dr. M. BODANSZKY.

Bioassay. For the study of each fraction 2 groups were formed from 5 anterior hypophyses of adult male Wistar rats of 150–180 g body weight: group A and group B.

Group A: 5 half-anterior hypophyses were incubated for 3 h 30 min in 16 ml of Krebs-Ringer solution at 38°C. Group B: 5 corresponding half-anterior hypophyses were incubated for 3 h 30 min in the same quantity of Krebs-Ringer solution at 38°C, but together with synthetic arginine vasotocin, as previously for sheep pineal powder and sheep pineal extract[4,5].

For group A and for group B the half-anterior hypophyses were removed after incubation; the incubation solution, obtained after centrifugation, was injected s.c. into immature 21-day-old female Swiss mice of 8–10 g body weight which had been sensitized just before the first injection with 0.25 IU of human chorionic gonadotrophin (HCG). Each mouse received a quantity of the incubation solution corresponding to the secretion of 1 half-anterior hypophysis in 5 injections in 3 days. Autopsy was carried out 18 h after the last injection.

We compared the average value of the ovary weights and the average value of the uterine weights of group A with the average values of group B. Standard errors of the means were calculated. A histological study of the ovaries of both groups completed this bioassay.

MOSZKOWSKA[4] has shown previously that the action of the antigonadotrophic activity of the pineal body acts on the secretion of the anterior-hypophysis and not on the receptors.

We repeated this experiment with arginine vasotocin. The incubation solution of 5 half-anterior hypophyses alone was mixed, after an incubation time of 3 h 30 min,

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Reactions of immature mice, sensitized with 0.25 IU of HCG, after the injection of each animal with the incubation solution of a half-anterior hypophysis of a male rat, whether alone (group A), incubated together with arginine-vasotocin (group B_1 and B_2), or incubated alone and mixed afterwards with arginine vasotocin (group C). The animals of group D were injected with 0.25 IU of HCG

Experiment	Group	Treatment	No. of animals injected	Mean weight of an ovary (mg) + S.E.	Maximum diameter of the follicles μ	Mean weight of the uterus (mg) $\pm$ S.E.
I	A	hyp.[a] incubated alone	5	8.63 ± 0.66	520 (1/5)[b] and 475 (5/5)[b]	102.70 ± 4.67
	B_1	hyp. incubated with 4 IU of arginine vasotocin/half-anterior hypophysis	5	6.28 ± 0.38 $P < 0.05$	475 (5/5)	48.30 ± 3.78 $P < 0.01$
	B_2	hyp. incubated with 10 IU of arginine vasotocin/half-anterior hypophysis	5	7.00 ± 0.85 n.s.	427 (1/5); 380 (5/5)	51.11 ± 4.94 $P < 0.01$
II	A	hyp. incubated alone	5	6.72 ± 0.65	475 (1/5); 427 (5/5)	74.50 ± 7.36
	C	hyp. incubated alone; the incubation solution corresponding to a half-anterior hypophysis is mixed afterwards with 2 IU of arginine vasotocin	5	5.23 ± 1.09 n.s.	475 (1/5); 427 (2/5) 380 (5/5)	58.98 ± 2.75 $P \leqslant 0.05$
III	A	hyp. incubated alone	5	7.15 ± 0.54	475 (10/10)	84.83 ± 9.43
	C	hyp. incubated alone; the incubation solution corresponding to a half-anterior hypophysis is mixed afterwards with 2 IU of arginine vasotocin	10	6.50 ± 0.36 n.s.	475 (1/10); 427 (1/10) 380 (10/10)	52.26 ± 3.84 $P < 0.01$
IV	D	injected with 0.25 IU HCG	5	3.96 ± 0.45	$\leqslant 280$ (5/5)	24.74 ± 1.90

[a] hyp., half-anterior hypophysis of an adult male rat of 150–180 g body weight. [b] 1/5, one of the five mice observed has ovaries containing follicles with a diameter of 530 μ. 5/5, all mice have ovaries containing follicles with a diameter of 475 μ.

with arginine-vasotocin, and this solution was injected into 5 mice, as previously described (group C).

Results. The Table summarizes the results of our experiments with synthetic arginine vasotocin, performed with immature mice as test animals.

Discussion. The Table shows that arginine vasotocin, whether incubated with half-anterior hypophyses for 3 h 30 min or mixed with the incubation solution of these organs from male rats incubated alone, can diminish significantly the influence of that incubation solution on the uterus weight when injected into immature mice.

As REVIERS and MAULÉON[6] have shown, the bioassay of IGARASHI and MCCANN[7], which is based on the uterus weight of immature mice, is not very specific for measuring the quantity of follicle stimulating hormone (FSH) in a solution. Therefore the influence of a solution on the uterus weight has to be interpreted cautiously. We studied the weight of the uterus and the weight of the ovaries and completed our bioassay by a histological examination of the ovaries.

Our results show further that only in group B1 does the weight of the ovaries differ significantly from the weight of the ovaries of the animals in group A. In group B1 we have also found that the maximum diameter of the follicles is a little smaller than that of the animals of group A. However, the maximum diameter of the follicles of the ovaries of group B_1 is much larger than that of the animals sensitized with 0.25 IU of HCG. Previously we have shown that fresh rat pineal bodies[4], acetone-dried sheep pineal powder[4] and a fraction F3 of a sheep pineal extract after gel-filtration on Sephadex G-25 (Fine)[5] can diminish or inhibit the secretion of the anterior hypophysis into the incubation medium, so that the ovaries of the test animals show a growth of the follicles comparable with that of the ovaries of the mice sensitized with HCG only. On the contrary, the ovaries of the mice injected with the incubation solution of anterior hypophysis alone show a certain growth of the follicles. The effects observed in group B1, group B2 and group C are comparable. Therefore we believe arginine vasotocin to act on the gonads or on the gonadotrophic hormone(s) and not on the secretion of the anterior hypophysis in vitro, as we have observed in in vitro experiments with acetone-dried sheep pineal powder[4,8,9].

Résumé. L'arginine vasotocine est capable d'inhiber la réponse de la souris impubère à la stimulation gonadotrope, mais ne semble pas agir directement in vitro sur l'excrétion hypophysaire, comme le fait le facteur inhibiteur épiphysaire présent dans la poudre totale et dans la fraction F3, obtenu après filtration d'un extrait épiphysaire sur gel Séphadex G-25 (Fine).

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The Cortisol Metabolism of Human Pulmonary Tumour Tissue

Malignant cells are known to metabolize cortisol. Cells originating from lymphosarcoma are able to oxidize or reduce at C-11 and C-20[1]. BURTON[2] reported on similar results in his mouse and rat experiments. We too were able to demonstrate steroid metabolization in different transplantable rat tumour tissues[3]. HALEY[4] investigated the corticoid metabolism of cancer tissue in mammilary cancer and observed different steroid metabolisms of individually different efficiencies without establishing a morphological correlation. To the best of our knowledge the steroid hormon catabolitic activity in human malignant pulmonary tumour has not yet been studied.

Material and methods. Sections of a surgically removed tumour were kept on ice and processed within 1 h. 1 g of the apparently intact tissue was cut up and the slices were placed into 30 ml of Krebs-Ringer bicarbonate solution. 1 μC 1–2 ^{3}H cortisol in 0.1 ml of alcohol solution was added (1 mC/mg cortisol), and was incubated at 37°C under a continuous stream of $CO_2 + O_2$ gas mixture. 1 g of the same patient's pulmonary tissue was incubated as a control. The medium was extracted with chloroform (3 × vol) evaporated in vacuo. The extract was first purified[5] and the steroids separated in a Bush B5 solution system. To ensure identification, in parallel test acetylation was performed for 24 h with pyridine and acetaldehyde, and the material was run beside cortisol acetate and cortisone acetate standards in a Bush 3 system. The activities of the paper strips (cut into 1 cm segments) were measured with the 'Packard Tricarb' liquid scintillation spectrometer. Histological investigation was carried out on every tumour.

Results and discussion. In the Table the cases are arranged according to the intensity of observed metabolization. This is shown in the third column. In the first 3 cases no metabolization was observed; transformation ratio increased rising from the fourth to the thirteenth case. With No. 14 a considerable quantity of polar fraction was obtained. A transformation product of this kind

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