vascular alterations by themselves can maintain and aggravate hypertension and make it independent from central mechanisms. Thus, what possibly commenced as a disturbance in the control of a central 'trigger' mechanism can end as self-perpetuating hypertensive disease.

Zusammenfassung. Zerstörung zentraler adrenerger Neurone durch Injektion von 6-Hydroxydopamin in einen Seitenventrikel verursachte bei normotonen und genetisch hypertonen Ratten lediglich eine geringe und vorübergehende Blutdrucksenkung und beeinflusste die DOCA- und renale Hypertonie überhaupt nicht. Im Gegensatz dazu verhinderte 6-Hydroxydopamin die Entwicklung der DOCA- und renalen Hypertonie, wenn es 7–10 Tage vor deren Induktion in einen Seitenventrikel injiziert wurde. Bei genetisch hypertonen Ratten, bei denen eine allmähliche Blutdrucksteigerung schon bald nach der Geburt einsetzt, unterbrach intraventrikulär injiziertes 6-Hydroxydopamin die weitere Entwicklung der Hypertonie. Die Resultate weisen auf die Bedeutung zentraler adrenerger Neurone für die Pathogenese verschiedener experimenteller Hypertonieformen hin.

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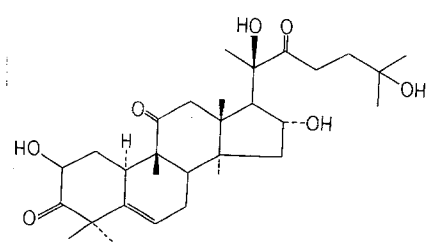
Antifertility Activity of Dihydroelatericin A in the Female Mouse

Dihydroelatericin A (I) is a synthetic derivative of elatericin A (also known as cucurbitacin D), a naturally occurring tetracyclic triterpene obtained from *Ecballium elaterium*[1] and several other species of the Cucurbitaceae plant family[2]. Certain cucurbitacins have been previously shown to possess potent carcinostatic activity[3,4].

During a subacute toxicity study performed on dihydroelatericin A, some effects were observed in the histological preparations of the female reproductive organs. These findings induced us to study the effects of the compound on the fertility of female mice.



I

Materials and methods. Dihydroelatericin A is a water-insoluble compound and therefore was administered per os as a suspension of 0.5% carboxymethyl cellulose (CMC) in saline. Adult female mice of proved fertility were caged in colonies 4–10 mice each and treated daily for 6 to 12 days with dihydroelatericin A at different dose levels from 10–100 mg/kg. After this pretreatment of 6–12 days, 2–5 adult males were added to each cage and daily treatment of the females continued thereafter for 7 to 14 days. Upon discontinuation of the dihydroelatericin A treatment, cohabitation was maintained for 120 days. Control female mice were treated in the same manner with 0.5% CMC in saline and mated. The number of pregnancies in each group was recorded.

The uterotrophic activity of the compound at different dose levels from 10–100 mg/kg was tested in groups of 6 immature mice according to the Rubin[5] assay.

The female reproductive organs were taken from 2 females of every treatment group 24 h after the last treatment and also after recovery period of 4 weeks and fixed in 10% formalin. The uteri of the immature mice were weighed 24 h after the treatment and fixed as above. The organs were stained by haematoxylin-Eosin and by PAS stain.

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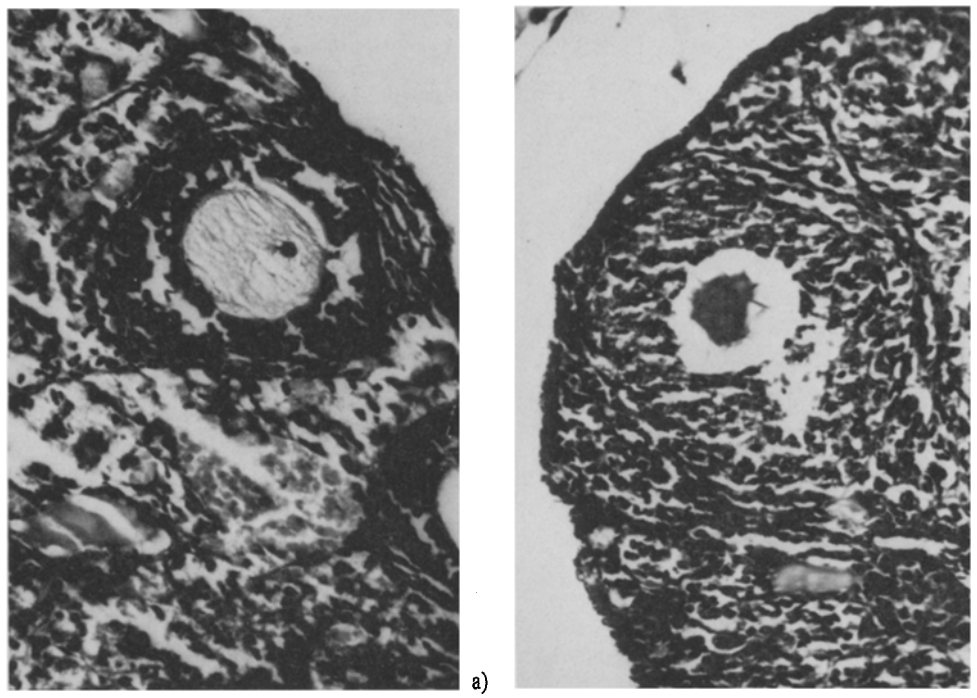
Table I. Effect of dihydroelatericin A on the fertility of mice

Group No.	Daily dose (mg/kg per os)	No. of animals in groups	Fertile mating				Not fertile after 120 days
			During treatment	After treatment (days) 1–30	30–60	60–120	
I	0	10	8/10	2/10	0/10	0/10	0/10
II	20	10	6/10	4/10	0/10	0/10	0/10
III	40	10	7/10	3/10	0/10	0/10	0/10
IV	100	40	1/40	5/40	10/40	13/40	11/40
Significance[a] of difference between I and IV (p)			<0.001	<0.001	<0.01	0.1–0.05	0.1–0.05

[a] The probability that the observed differences between the compared groups was due to chance, was calculated according to Student's t-test.



Photomicrographs of sections stained by hematoxylin eosin. × 1000. a) Ovary of an untreated female mouse. b) Ovary of dihydroelatericin A treated female mouse (100 mg/kg for 28 days).

Results. Table I shows the fertile matings that occurred in the dihydroelatericin A treated mice. At the high doses dihydroelatericin A was contraceptive and almost no pregnancy in all treated groups. Following withdrawal of administration and during a period of observation of 120 days thereafter, fertility returned in 28 of 40 animals, whereas 11 out of 40 remained unfertile.

Table II shows the activity of dihydroelatericin A on the immature mouse uterus. A remarkable increase in uterus weight was recorded upon administration of a total of 50 mg/kg of compound or more. A 10-fold dose (500 mg/kg) did not produce additional increase in organ weight. No antagonistic or synergistic effect of dihydroelatericin A on the action of estrogen on uterus growth was found. Inspection of the immature uteri showed minor congestions in the treated mice.

In the mature females, clear inhibition of ovulation has been determined by microscopic examination of the ovaries. Many atretic follicles were present in all ovaries of the mice treated with dihydroelatericin A at a dose of 100 mg/kg for at least 2 weeks (Figure). The failure to form corpora lutea was also noted. The glands of the uteri of these mice were found to be in a resting phase, some showing a process of hyperplasia of the serosa.

Pregnant mice (a group of 10), when treated for 5 days with 100 mg/kg dihydroelatericin A from the 8th day of pregnancy, did not show any effect on the developing animals foetus, and the viability of litters was unaffected. None of the litters showed congenital malformations at macroscopic inspection.

The contraceptive mechanism of action appears to be by prevention of ovulation. It should be noted, however, that this effect is to a large extent reversible for the animals treated with a daily dose of 100 mg/kg body weight when under observation for up to 120 days following withdrawal of administration of the substance.

Although dihydroelatericin A had no tumor growth inhibitory effect on a variety of transplanted tumors studied[4], an enhancing effect on the growth of mammary adenocarcinoma MMC_1A of female Swiss mice had been found, whereas when given to ovariectomized mice, it produced an inhibitory effect on the growth of the tu-

Table II. Effect of dihydroelatericin A on uterus weight of immature mice

Compound	Dose (mg/kg per os) daily	total	No. of animals	Mean body weight at end of exp. (g ± SD)	Mean uterus weight (mg ± SD)	Ratio (mg uterus wt./g body weight)
Saline	0	0	6	9 ± 2.1	8.3 ± 2.7	0.9
Dihydroelatericin A	10	30	6	7.8 ± 1.7	10 ± 1.3	1.2
Dihydroelatericin A	10	50	6	10.5 ± 1.72	16.4 ± 2.5	1.57
Dihydroelatericin A	20	80	6	8.4 ± 1.3	15 ± 3.4	1.7
Dihydroelatericin A	100	500	6	13.5 ± 1.18	21 ± 8.6	1.5
Estradiol	0.05	0.1	6	8.1 ± 0.9	45 ± 2	5.5
Estradiol + Dihydroelatericin A	0.05 + 20	0.1 + 80	6	7.5 ± 1	39 ± 2.1	5.7

mor[6]. These observations together with the present report indicate that this compound is capable of interfering in some way with endocrine mechanisms in the female reproductive system.

Résumé. Dihydroelatericine A administré par voie buccale à des souris femelles au moins pendant deux semaines, empêche la fertilisation. L'éxamen histologique des ovaires démontre que la substance prévient l'ovulation.

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Ultrastrukturelle Untersuchungen der juxtaepiphysären Kapillaren nach Perfusionsfixation

Während die knorpelige Epiphyse eines Embryos noch von zahlreichen gefässführenden Knorpelkanälen durchzogen wird, ist mit dem Einsetzen der enchondralen Ossifikation der hyaline Epiphysenknorpel bei den Säugetieren gefässlos (Brookes[1]). Bei den Vögeln hingegen wird der Wachstumsknorpel der langen Röhrenknochen von sog. Knorpelkanälen in seiner ganzen Länge durchbohrt (Lutfi[2]). Diese Knorpelkanäle enthalten Kapillaren, die mit den juxtaepiphysären Kapillaren in Verbindung stehen.

Der Einsprossungsprozess der juxtaepiphysären Kapillaren in den Epiphysenknorpel war Ziel wiederholter licht- und elektronenmikroskopischer Untersuchungen (Irving[3]; Anderson und Parker[4]; Schenk et al[5,6]; Brookes[1]). Nach routinemässiger Gewebeentnahme und Immersionsfixation wurden in den juxtaepiphysären Kapillaren grosse Endothellücken beschrieben, durch welche zahlreiche Erythrocyten in die eröffneten Lakunen ausgetreten waren (Anderson und Parker[4]; Schenk et al.[5,6]). Es war deshalb von Interesse, die Ultrastruktur der Epiphysen-Metaphysengrenze nach Perfusionsfixation zu untersuchen.

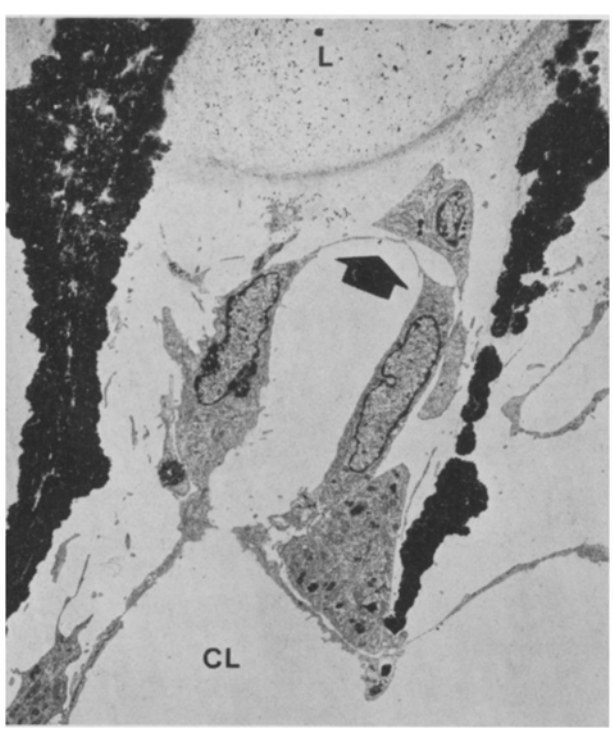

Fig. 1. Kapillarsprosse aus einer juxtaepiphysären Kapillare (Pfeil) dringt in Lakune (L) zwischen zwei mineralisierte Knorpelsepten vor. Beachte das intakte Endothel ohne Erythrocyten-Extravasat nach der Perfusionsfixation. CL = Kapillarlumen. Perfusionsdruck: 140 cm H_2O. ×3800.

Material und methodik. Für die Untersuchung wurden 6 männliche Wistar-Ratten (mittleres Gewicht 80 g) verwendet. Die Tiere erhielten eine Altromin-R-Standard-Diät sowie Trinkwasser ad libitum. Die Perfusion selbst erfolgte weitgehend nach Angaben von Forssmann et al.[7]. Da eine gute Kühlung der Perfusionslösung für die Gewebeerhaltung wesentlich schien, wurden in Abänderung der von den genannten Autoren beschriebenen Anlage das Kühlbad und die Vorrichtung zur Sättigung der ersten Spüllösung mit Sauerstoff unmittelbar vor die Ansatzstelle für den Mikroschlauch gesetzt. Ein Dosenmanometer mit Reiberhahn wurde am Kühlbehälter angebracht und erlaubte so, sowohl den Blutdruck der Versuchstiere bei Perfusionsbeginn zu messen als auch den gewünschten Perfusionsdruck einzuregulieren.

Die Narkose der Tiere erfolgte durch i.p. Verabreichung von Nembutal (ca. 60 mg/kg KG). Procedere: Ein heparinisierter PVC-Mikroschlauch PE 90 wurde in die Aorta descendens eingeführt. Der gemessene Blutdruck betrug bei den Tieren im Durchschnitt ca. 100 bis 110 cm H_2O. Eine vorher angelegte Ligaturschlinge um die Aorta descendens wurde angezogen und der Mikroschlauch in die Arteria iliaca communis vorgeschoben. Während 5 min wurde mit einer Ringer-Procain-Lösung nach Forssmann et al.[7] (4–8°C; ph = 7,4; 350 mOsm) in zwei Varianten gespült: bei 3 Tieren bei einem Druck von 100 cm H_2O; bei 3 Tieren bei einem Druck von 140 cm H_2O. In einem weiteren Schritt wurde sodann mit einer 2,5%igen Glutardialdehydlösung (4–8°C; pH 7,4; 0,1 molar; 350 mOsm) bei einem Perfusionsdruck von 60–80 cm HO_2 während 30 min fixiert. Nach nochmaliger Durchspülung mit s-Collidin-Waschpuffer (400 mOsm) war die Perfusionsfixation der Tibiaepiphysenfugen beendigt. Daran anschliessend erfolgte die Entnahme der Tibiaepiphysenfuge

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