Table II. LD_{50}-values (mmol/kg, 95% confidence limits in parentheses), calculated on mortalities within 24 h and within 7 days

Compound	Species	24 h		7 d	
		p.o.	i.v.	p.o.	i.v.
Pyrazole	Rat	21 (20–23)	19 (18–21)	17 (14–20)	15 (12–19)
	Mouse	22 (21–24)	21 (20–23)	6.0 (3.6–9.4)	5.5[a] —
4-Methylpyrazole	Rat	7.9 (6.1–10.1)	3.8 (3.5–4.3)	6.5 (5.9–7.1)	3.8 (3.5–4.3)
	Mouse	7.8 (6.6–9.2)	3.8 (3.2–4.6)	7.8 (6.6–9.2)	3.8 (3.2–4.6)

[a] Graphical estimation.

deposits of haemosiderin. In the spleen the deposition of iron was significantly and in the bone marrow slightly denser in rats of the high dose group than in rats of the control one.

Discussion. The acute toxicity of pyrazole has previously been estimated in rats and mice after oral and intraperitoneal administration[3,4,9]. The results of the present study mainly agree with these earlier observations when comparable. In contrast to 4-methylpyrazole, pyrazole showed a greater acute toxicity after 7 days than after 1 day, especially manifested in mice. The observation indicates a delayed toxicity of pyrazole, which is in agreement with findings by LELBACH[4]. The delayed toxicity may be related to the essential differences between 4-methylpyrazole and pyrazole as indicated by the results of the subacute investigations. No lesions were found after treatment with the former compound, whereas the latter caused several changes. It was, thus, found that pyrazole induced a hyperplasia of the thyroid, a hepatomegaly, an atrophia of the testis and accessory glands, an anaemia and a depression of the bone marrow. Some of these alterations were also observed by WILSON and BOTTIGLIERI[3]. It is probable that organic lesions are involved in the mechanism of the delayed toxicity of pyrazole.

The present study shows that repeated oral administration of 4-methylpyrazole to rats is well tolerated in doses which inhibit the alcohol dehydrogenase efficiently[2]. This is in contrast to the unsubstituted pyrazole which in equimolar doses causes severe toxicity. Since the 4-methylpyrazole is several times more potent than pyrazole, it seems to be the compound of choice for pharmacological and metabolic studies of inhibition of ethanol metabolism.

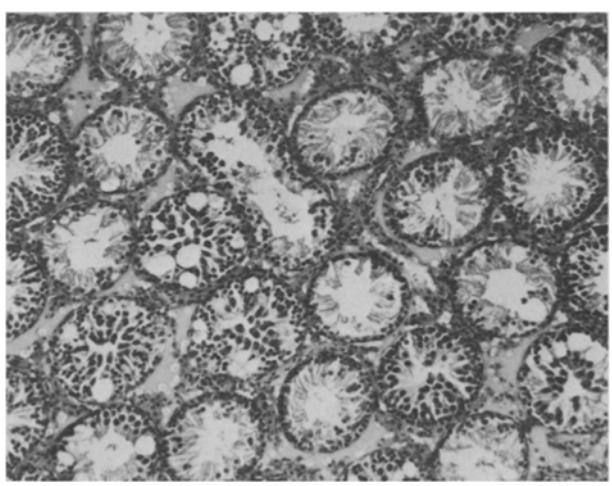

Fig. 2. Testis of a rat after oral administration of 1.2–2.4 mmol/kg pyrazole for 4 weeks. The testicular tissue shows an atrophia with degenerative changes of the seminiferous epithelium. Haematoxylin and eosin. Magnification ×75.

Zusammenfassung. Subchronische Gaben von Pyrazol, nicht dagegen von 4-Methylpyrazol, führen bei Ratten und Mäusen zu toxischen Läsionen.

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Central Adrenergic Neurones and the Initiation and Development of Experimental Hypertension

Established arterial hypertension in both animals and human patients is characterized by an increased systemic flow resistance[1,2]. Mechanisms operating at the pre- and postjunctional site of the vascular neuroeffector system have been causally related to this elevated resistance. At the prejunctional site an increase of sympathetic vasoconstrictor activity (A) has been suggested[3,4]. Postjunctionally several processes have to be considered: The occurrence of humoral vasoconstrictor factors (B)[5,6], a change in the sensitivity of the individual vascular smooth muscle cells (C)[7,8] to an otherwise normal vasoconstrictor input or an increased reactivity of the blood vessels (D) due to an adaptive structural change of the vessel wall, i.e. an increase of the wall/lumen ratio[2,9]. Since D is the result of maintained high blood pressure, it seems to be of no importance for the initiation of the hypertensive state. However, A, B and C would be able to set in motion the process leading to hypertension. It may be assumed that the appearance of these factors (A, B, C) is triggered off by a hitherto unknown mechanism. A possible involvement of central adrenergic neurones in hypertension is indicated by a decrease in noradrenaline turnover in the brainstem of rats with an established DOCA/NaCl hypertension[10] and by an increase in norad-

renaline turnover in the hypothalamus and thoracolumbar region of the spinal cord in rabbits with neurogenic hypertension[11].

We have studied the effect of the destruction of central adrenergic neurones by 6-hydroxydopamine (6-OH-DA)[12,13] on 3 types of experimental hypertension in the rat. Renal and DOCA/NaCl hypertension was induced in 7-week-old male rats from a closed randomized colony (Wistar descent) according to methods previously described[14]. Both types of experimental hypertension were fully developed 6–7 weeks later. Furthermore, spontaneously (genetic) hypertensive rats (SHR)[15] were included in our experiments. The systolic blood pressure (BP) was measured in all conscious rats by a tail cuff method[16].

2–3 injections of 6-OH-DA (each 250 µg) were given at 2 day intervals into the left lateral brain ventricle under halothane anaesthesia using a David Kopf stereotaxic instrument. The compound was dissolved in 0.9% w/v sodium chloride solution to which ascorbic acid (1 mg/ml) was added and which was gassed with nitrogen. The injection volume was 10 µl. Control animals were injected intra-ventricularly with the same volume of the vehicle solution. At the end of the experiment tyrosine hydroxylase activity[17,18] and noradrenaline[19,20] content were measured in the hypothalamus, medulla oblongata and the residual parts of the brain.

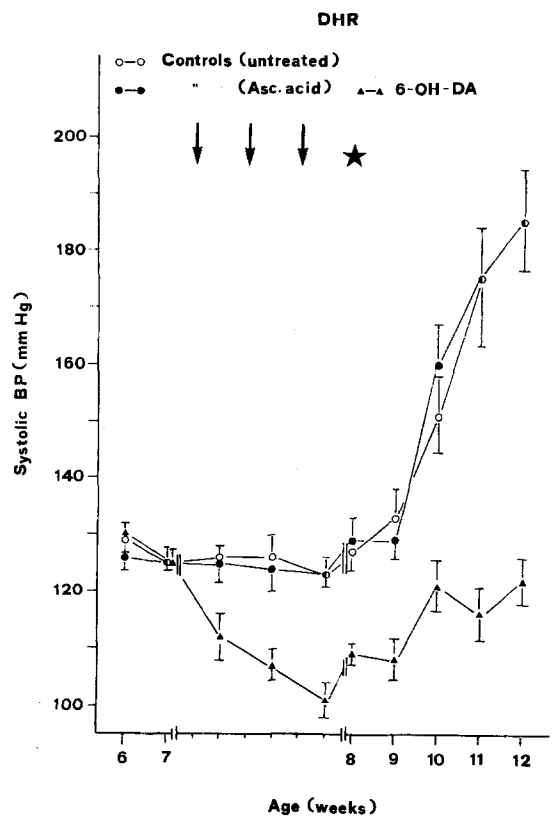

Fig. 1. Prevention of the development of DOCA/NaCl hypertension in the rat by 3 intraventricular injections of each 250 µg 6-hydroxydopamine (arrows). The asterisk indicates the procedures (unilateral nephrectomy, implantation of DOCA tablets, 0.9% w/v sodium chloride solution as drinking water) to induce hypertension. Controls did either not receive an intraventricular injection (= untreated) or were intraventricularly injected with the vehicle solution (= Asc. acid) of 6-hydroxydopamine (n = 12 for each group).

6-OH-DA (3 × 250 µg) injected intraventricularly into 12–14-week-old normotensive rats (mean BP = 128 mm Hg) and SHR (192 mm Hg) produced a moderate fall in BP by 20–25 mm Hg. However, 5–7 days after the last injection of 6-OH-DA the BP had returned to the pre-injection level. In DOCA/NaCl hypertensive rats (DHR) (215 mm Hg) and renal hypertensive rats (RHR) (223 mm Hg) the intraventricular administration of 6-OH-DA was followed by only a very small fall in BP. The determination of the noradrenaline content in the brain 2 weeks after the last injection of 6-OH-DA revealed a decrease to 20% of that of the controls (= 100%) in the hypothalamus, to 40% in the medulla oblongata and to 12% in the residual parts of the brain for normotensive and hypertensive rats. The corresponding values for tyrosine hydrosine hydroxylase activity were 45, 50 and 20%, respectively. Thus, a condiserable destruction of central adrenergic structures by 6-OH-DA did not affect established renal and DOCA/NaCl hypertension and caused only a transient moderate fall in BP in normotensive rats and SHR. This is in agreement with recently published data[21].

Quite different results were obtained when 6-OH-DA was injected into the lateral brain ventricle before the induction of renal and DOCA/NaCl hypertension or during the development of spontaneous hypertension in 7-week-old SHR. Figure 1 shows the prevention of DOCA/NaCl hypertension by prior intraventricular injections of 6-OH-DA. After a transient fall following the 3 successive administrations of 6-OH-DA, the BP returned to normal without any further rise. Both in the untreated and the vehicle-injected controls the BP increased in a virtually identical manner during the same observation period. Noradrenaline content and tyrosine hydroxylase activity measured in several parts of the brain at the end of the experiment were lowered to a similar extent as described above for rats which were injected with 6-OH-DA at a more advanced age. The noradrenaline content of the heart was not affected by the intraventricular injections

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of 6-OH-DA, indicating the absence of a peripheral sympathectomy. Immediately after the intraventricular injections of 6-OH-DA the animals did not gain weight. However, 1 week later a normal development set in again and at the end of the 6-week experiment the animals did not differ from untreated controls with regard to the nutritional state. In another series of experiments we studied the effect of intraventricularly administered 6-OH-DA on the development of renal hypertension. Here again the rise in BP could be prevented by the prior treatment with 6-OH-DA and the results resemble closely those depicted in Figure 1 for DHR.

In SHR BP starts to rise 2–3 weeks after birth. At the age of 6–7 weeks the BP varies between 160–170 mm Hg (Figure 2) and in 12–14-week-old SHR it has reached a plateau which for our colony of SHR is 180–210 mm Hg. Figure 2 shows that 2 intraventricular injections of 6-OH-DA in 7-week-old SHR cause a fall in BP to approximately 140 mm Hg. There is no further rise in BP in the treated animals up to the 12th week, while vehicle-injected controls exhibit the usual development of spontaneous hypertension during the same observation period (Figure 2). Thus, 6-OH-DA injected intraventricularly in 7-week-old SHR is able to interrupt the further development of this type of hypertension.

In a second set of experiments with DHR and RHR which confirmed the results on BP development described above, vascular reactivity was compared in 6-OH-DA treated and control animals. A modified isolated mesenteric artery preparation[22] was used for these investigations. The mesenteric arteries of vehicle-injected rats which developed DOCA/NaCl or renal hypertension showed on increased reactivity to the vasoconstrictor effect of noradrenaline as was previously described for several types of hypertensive rats[9, 22, 23]. As compared with normotensive controls the dose-response curves for noradrenaline of the hypertensive animals were steeper and displayed a higher maximum indicating an adaptive structural change of the vessel wall due to the hypertension[2, 9]. The corresponding dose-response curves obtained in mesenteric artery preparations from 6-OH-DA treated rats which had not developed DOCA/NaCl or renal hypertension were virtually identical with those of normotensive controls.

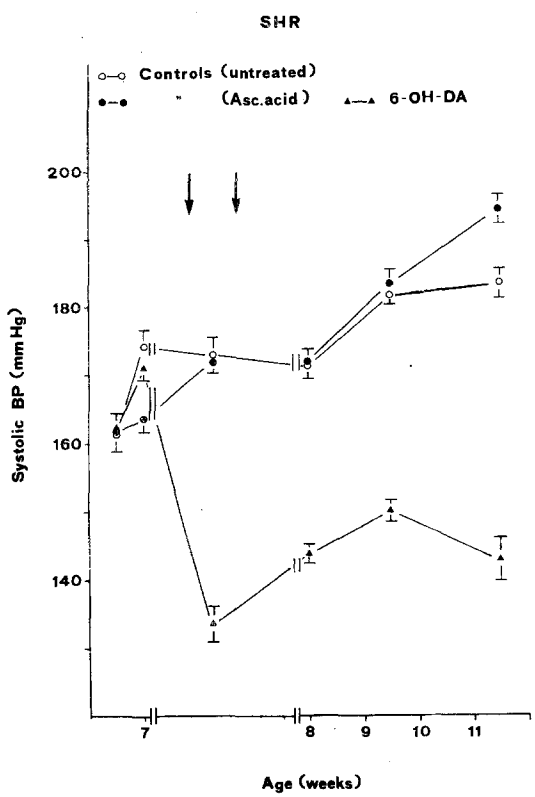

Fig. 2. Interruption of the further rise in blood pressure in 7-week-old spontaneously hypertensive rats (SHR) by 2 intraventricular injections of each 250 µg 6-hydroxydopamine (arrows) (n = 12 for each group).

The differential effect of 6-OH-DA injected intraventricularly before the induction or during the development of hypertension as compared with its administration in established hypertension has led us to suggest a centrally located 'trigger' mechanism for the initiation of the 3 types of experimental hypertension under investigation. This mechanism must be under the control of noradrenaline and/or dopamine since 6-OH-DA is known to destroy rather selectively noradrenergic and dopaminergic but, for instance, not serotoninergic structures in the brain[12]. It is interesting to note that the nucleus of the solitary tract in which the majority of the fibres of the carotid sinus nerve terminates[24, 25] is densely supplied with noradrenergic nerve terminals in the rat[26]. Therefore, an involvement of noradrenergic neurones in the modulation of the baroreceptor reflexes seems to be conceivable.

It is tempting to speculate that an increase in sympathetic vasoconstrictor activity in the periphery is set in motion by the proposed central 'trigger' mechanism. Cardiovascular response patterns due to an increase of sympathetic nerve activity can be elicited by activation of the brain stem reticular formation. One of the best-known is the defence-alarm reaction which can be induced by stimulation of the anterior hypothalamus and which – when permanently elicited – can lead to an elevated BP[4]. However, the 'trigger' mechanism could also be related to processes which are endocrine in nature and governed by central adrenergic neurones. Furthermore, it has to be considered that destruction of the central adrenergic neurones may prevent the rise in blood pressure even if the latter were originally caused by a disturbance or disease of some other organ.

So far, no information can be given as to the localization of the 'trigger' mechanism in the central nervous system, since 6-OH-DA when injected into the lateral ventricle of the brain distributes widely throughout the central nervous system and affects noradrenergic and dopaminergic neurones in all parts of the brain and the spinal cord[13].

The failure of intraventricular 6-OH-DA to lower BP in established hypertension seems to confirm the role of some central adrenergic neurones as having merely a 'trigger' function. It has been shown that maintained high BP induces adaptive structural changes in the arterial wall[2, 9]. The media becomes thicker and the ratio of wall thickness to vascular lumen increases. These changes, in turn, considerably augment the reactivity of the blood vessels to vasoconstrictor stimuli[2, 9], an observation which could be confirmed in our experiments on the isolated mesenteric artery preparation (see above). Therefore, the

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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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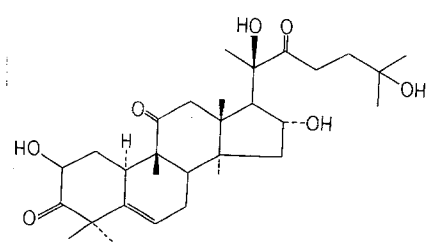
Antifertility Activity of Dihydroelatericin A in the Female Mouse

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.



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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.