Arachidonic acid was dissolved in sterile, pyrogen-free 0.9% sodium chloride solution and just before i.c.v. injection the solution was passed through a millipore filter. The antagonists were injected either i.v. (cyproheptadine, phenoxybenzamine and pimozide), or s.c. (indomethacin) in most experiments before the i.c.v. administration of the agonists. The time-interval was 30 min for all the drugs. The 6-OH-DA was dissolved in sterile, pyrogen-free 0.9% sodium chloride solution containing 1 mg/ml ascorbic acid. Just before injection the solution was adjusted to pH 5.0 with MNaOH and passed through a millipore filter. In the case of PCPA, it was administered orally in gelatine capsules in a dose of 300 mg/kg, a similar dose being given 24 h later.

Doses are expressed per kg b.wt. For cyproheptadine (Merk, Sharp & Dohme), phenoxybenzamine (Smith, Kline & French), and 6-hydroxy-dopamine (6-OH-DA) (Biotec, Sweden) they refer to the hydrochlorides; for pimozide (Janssen, Belgium), indomethacin (Merk, Sharp & Dohme), and (para-chloro-phenyl-alanine) (PCPA) (Sigma) to the compound itself; and for arachidonic acid (Sigma) to its sodium salt.

Temperatures were measured using a thermistor probe introduced 12 cm into the rectum and connected to an Elektrolaboratoriet' apparatus. The animals were placed in restraining cages at least 2 h before the beginning of the experiments. The room temperature was 22 ± 2 °C. Statistical significance was assessed with the classical t-test.

Results. Hyperthermia was induced in the rabbit by arachidonic acid, 100 µg/kg i.c.v. pimozide (1 mg/kg) had no significant inhibitory effect on arachidonic acid hyperthermia, whereas phenoxybenzamine (1 mg/kg) and cyproheptadine (3 mg/kg) abolished it ($p < 0.05$), as shown in figure 1.

Rabbits were injected on days 1,4 and 7 with 6-OH-DA (500 µg/kg i,c.v.). Following the 1st dose, there was an immediate rise of temperature in all animals. After the second dose, there was a considerably smaller rise in body temperature. After the 3rd dose, there was either no effect or a very small rise in deep body temperature.

The hyperthermia produced by arachidonic acid (100 µg/kg i.c.v.) was reduced by 40% after the animals had been treated with 6-OH-DA (figure 2), but in PCPA-treated animals (300 mg/kg), there was no decrease. Indomethacin (10 mg/kg) attenuated the rise induced by arachidonic acid by 80%, as shown in figure 3.

Discussion. The decrease in arachidonic acid hyperthermia after 6-OH-DA and the failure of PCPA to reduce this rise in temperature argues in favour of the view that arachidonic acid hyperthermia may be partly mediated by noradrenaline, though the direct action of arachidonic acid cannot be excluded, as there was only 40% reduction in arachidonic acid hyperthermia. The antagonism of arachidonic acid hyperthermia by phenoxybenzamine indicates the involvement of central α-adrenoceptors. Cyproheptadine antagonism on arachidonic acid hyperthermia may be due to its inhibitory action on PGs synthesis[14]. The attenuation of arachidonic acid hyperthermia by indomethacin in vivo confirms the hypothesis of Vane[10], that anti-inflammatory substances inhibit PGs synthesis from their precursors.

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Chlorinated benzene induction of hepatic porphyria[1]

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Summary. 1,4-Dichlorobenzene and 1,2,4-trichlorobenzene were compared with hexachlorobenzene which is known to cause porphyria. Although hexachlorobenzene administration resulted in a manyfold increase in liver porphyrin levels and urinary excretion of porphyrins, the lesser chlorinated compounds did not do so.

The fungicide hexachlorobenzene has caused serious outbreaks of hepatic porphyria in Turkey as evidenced by cutanea tarda lesions and porphyrinuria[2,3]. This has been confirmed in a number of laboratory species including rats, rabbits, guinea-pigs and mice[3–8]. Humans are also exposed to the less chlorinated benzenes. 1,4-Dichlorobenzene is widely employed as a deodorizer and in mothballs. 1,2,4-Trichlorobenzene is used in solvents, oil additives and termite exterminators. All 3 of these compounds have been identified in both drinking water[9] and municipal waste water[10]. Despite numerous studies carried out with hexachlorobenzene, little work has been done with regard to the less chlorinated benzenes. Rimington and Ziegler[11] reported that feeding rats mono-, di-, tri- and tetrachlorobenzenes resulted in porphyria. However, since the doses used were very high (445–1140 mg/kg) and for short time periods (5–15 days), it was of interest to compare 1,4-dichlorobenzene and 1,2,4-trichlorobenzene with hexachlorobenzene at lower dose-levels for prolonged periods of administration.

Materials and methods. Groups of 5 rats (starting weights of 120–140 g) were used. Females were chosen because they are more susceptible to the porphyrinogenic effects of hexachlorobenzene[5,12]. Hexachlorobenzene, 1,2,4-trichlorobenzene and 1,4-dichlorobenzene were suspended

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Table 1. Effect of hexachlorobenzene p.o. on porphyrin production and excretion in female rats

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porhyrins (μg/24 h)
	30 days of administration		
0	6.2 ± 0.1[a]	348 ± 22[a]	1.4 ± 0.2[a]
50	9.9 ± 0.7[c]	1058 ± 197[b]	2.9 ± 0.7[b]
100	8.2 ± 0.5[b]	1730 ± 149[c]	2.0 ± 0.4[a,b]
200	9.4 ± 0.5[b,c]	1263 ± 280[b,c]	2.2 ± 0.2[a,b]
	60 days of administration		
0	7.0 ± 0.5[a]	1489 ± 746[a]	2.1 ± 0.3[a]
50	9.6 ± 0.2[b]	14100 ± 5790[b]	4.1 ± 0.7[b]
100	10.3 ± 0.4[b,c]	18700 ± 8690[b]	3.8 ± 0.7[a,b]
200	11.1 ± 0.6[c]	14400 ± 5210[b]	4.4 ± 0.5[b]
	90 days of administration		
0	8.2 ± 0.5[a]	9960 ± 1870[a]	2.2 ± 0.4[a]
50	10.7 ± 0.7[b]	38700 ± 10600[a,b]	36.2 ± 8.3[b]
100	10.4 ± 0.4[b]	317000 ± 127000[b]	43.3 ± 17.0[b]
200	12.4 ± 0.4[c]	149000 ± 33000[b]	30.4 ± 14.2[b]
	120 days of administration		
0	7.5 ± 0.4[a]	1200 ± 489[a]	2.0 ± 0.4[a]
50	11.5 ± 0.4[b]	199000 ± 59000[b]	236 ± 112[b]
100	12.5 ± 0.7[b]	318000 ± 65000[b]	357 ± 133[b]
200	11.3 ± 0.5[b]	317000 ± 63000[b]	194 ± 54[b]

[a–c] Values with same superscript are not significantly different ($p > 0.05$).

Table 2. Effect of 1,4-dichlorobenzene p.o. on porphyrin production and excretion in female rats

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (μg/24 h)
	30 days of administration		
0	6.8 ± 0.3[a]	246 ± 21[a]	1.5 ± 0.2[a]
50	6.6 ± 0.4[a]	269 ± 20[a]	1.9 ± 0.3[a]
100	7.0 ± 0.2[a]	251 ± 22[a]	1.4 ± 0.2[a]
200	8.0 ± 0.3[b]	276 ± 18[a]	1.4 ± 0.2[a]
	60 days of administration		
0	6.7 ± 0.3[a]	381 ± 20[a]	1.9 ± 0.4[a]
50	7.2 ± 0.4[a]	448 ± 17[a,b]	2.0 ± 0.4[a]
100	7.6 ± 0.3[a,b]	435 ± 19[a,b]	1.6 ± 0.2[a]
200	8.4 ± 0.3[b]	472 ± 30[b]	1.7 ± 0.3[a]
	90 days of administration		
0	6.8 ± 0.6[a]	541 ± 33[a]	0.9 ± 0.2[a]
50	7.0 ± 0.6[a]	527 ± 30[a]	1.0 ± 0.2[a]
100	6.6 ± 0.4[a]	555 ± 20[a]	1.3 ± 0.3[a]
200	7.2 ± 0.3[a]	548 ± 36[a]	0.8 ± 0.2[a]
	120 days of administration		
0	6.9 ± 0.2[a]	354 ± 10[a]	1.4 ± 0.2[a]
50	8.1 ± 0.3[b]	391 ± 18[b]	1.8 ± 0.4[a]
100	7.3 ± 0.6[a,b]	411 ± 9[b,c]	1.6 ± 0.4[a]
200	7.5 ± 0.1[a,b]	440 ± 8[c]	1.0 ± 0.2[a]

[a–c] Values with same superscript are not significantly different ($p > 0.05$).

Table 3. Effect of 1,2,4-trichlorobenzene p.o. on porphyrin production and excretion in female rats

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (μg/24 h)
	30 days of administration		
0	7.0 ± 0.3[a]	195 ± 9[a]	2.2 ± 0.4[a]
50	7.9 ± 0.2[a,b]	218 ± 7[a]	2.4 ± 0.4[a,b]
100	7.5 ± 0.7[a,b]	252 ± 16[b]	2.2 ± 0.4[a]
200	8.4 ± 0.4[b]	252 ± 8[b]	3.5 ± 0.3[b]
	60 days of administration		
0	6.3 ± 0.3[a]	304 ± 29[a]	1.4 ± 0.4[a]
50	7.4 ± 0.2[b]	305 ± 12[a]	1.5 ± 0.2[a]
100	8.1 ± 0.3[b,c]	371 ± 17[a]	1.8 ± 0.3[a]
200	8.5 ± 0.4[c]	361 ± 30[a]	1.9 ± 0.2[a]
	90 days of administration		
0	6.5 ± 0.4[a]	386 ± 8[a]	1.3 ± 0.2[a]
50	8.6 ± 0.5[b]	492 ± 36[a]	1.4 ± 0.1[a]
100	9.2 ± 0.3[b]	680 ± 63[b]	1.7 ± 0.3[a]
200	9.0 ± 0.4[b]	718 ± 40[b]	2.7 ± 0.4[b]
	120 days of administration		
0	8.4 ± 0.4[a]	330 ± 14[a]	1.7 ± 0.4[a]
50	8.6 ± 0.1[a]	396 ± 33[a,b]	2.3 ± 0.3[a]
100	9.6 ± 0.5[a]	469 ± 44[b]	2.2 ± 0.6[b]
200	8.9 ± 0.5[a]	462 ± 12[b]	1.6 ± 0.1[a]

[a–c] Values with same superscript are not significantly different ($p > 0.05$).

or dissolved in corn oil and administered p.o. daily at 50, 100 or 200 mg/kg for 30, 60, 90 or 120 days. Controls received corn oil (1% v/w).

Immediately after the last dose, the rats were placed in metabolism cages for 24 h urine samples. They were sacrificed, livers removed and portions prepared as $33^1/_3$% liver homogenates in 0.9% NaCl containing 0.5 mM EDTA and 10 mM tris buffer pH 7.2. Samples were precipitated with 0.3 M trichloroaceric acid and centrifuged. Urine samples or liver supernatants were passed through 'piggy-back' ion exchange columns[13] and separations carried out as described by Piper er al.[14]. After washing with water, the anionic column was eluted once with 2 ml of 1.0 N acetic acid and once with 2 ml of 0.2 N acetic acid and porphobilinogen (PBG) measured[15] and then with 8 ml of 1.5 N HCl and the porphyrins measured fluorometrically with excitation at 400 nm and emission at 600 nm. Coproporphyrin was the standard. The cationic column was eluted with 7 ml of 1 M sodium acetate and delta-aminolevulinic acid (ALA) determined[13]. In livers, only porphyrin content was measured. Comparisons among dose levels were made using Duncan's new multiple range test[16] with log transformation of the data when values varied by an order of magnitude or more.

Results and discussion. In agreement with the findings of others[2–8], hexachlorobenzene caused porphyria (table 1). After only 30 days, liver porphyrins were elevated even at 50 mg/kg. This increase was even more dramatic on a total liver basis since the liver was enlarged. After 120 days, the livers of the rats given 200 mg/kg day contained as much as 3 to 4 mg of porphyrin/liver. The controls varied more than with the other groups and may have been exposed to hexachlorobenzene from transfer cages during daily injections. No change was noted in the urinary excretion of ALA or PBG at any time. After 30 days the increase in porphyrin excretion was minimal but increases were greater after 60, 90 and 120 days. The response was dose-dependent.

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1,4-Dichlorobenzene had a low potential for causing porphyria (table 2). After 30 and 60 days there was a dose-dependent increase in the liver weight which is in agreement with previous results[17]. There were only slight increases in liver porphyrins even after 120 days. There were no differences between the treated animals and controls in the urinary excretion of ALA, PBG or porphyrins.

1,2,4-Trichlorobenzene also demonstrated a lack of ability to cause porphyria although the liver weight did increase (table 3). The increases seen in liver porphyrins were very small compared to those observed with hexachlorobenzene. Urinary excretion of ALA and PBG were not elevated at any time at any dose. Porphyrin excretion was only minimally increased at 200 mg/kg after 30 and 90 days.

The results, while confirming the ability of hexachlorobenzene to produce severe hepatic porphyria, indicate that 1,4-dichlorobenzene and 1,2,4-trichlorobenzene do not share this property. Even at doses up to 200 mg/kg for 120 days, there were no changes which would indicate a potential problem involved with the intake of these chemicals.

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The opposed influences of β-adrenergic stimulation and adenosine on the frequency-force relationship of isolated left atria of guinea-pigs

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Summary. The normal frequency-force relationship of left guinea-pig atria can be largely suspended when strong β-adrenergic stimulation by orciprenaline is antagonized by the negative inotropic effect of adenosine, so that contraction amplitude is nearly equal at an intermediate level over a wide range of stimulation rates. Curves obtained with a new method for recording continuous frequency-force loops are presented.

The dependence of myocardial contractility on heart rate or stimulus pattern, known as interval-strength relationship[1], certainly reflects basic processes of excitation-contraction coupling[2]. Accordingly, this relationship has been shown to be characteristically influenced by different types of intropic interventions, and further investigations should help to clarify open questions of both excitation-contraction coupling and inotropic mechanisms[1,2].

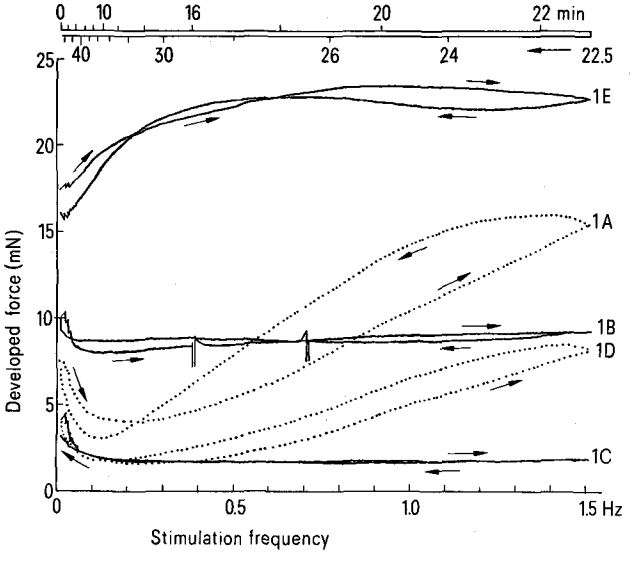

Fig. 1. 5 continuous frequency-force loops on the left atrium of a 190 g guinea-pig. In 45 min the frequency is changed from 0.007 Hz to 1.5 Hz and back to 0.007 Hz (time scale above the panel). Resting force 7.5 mN; 32 °C; 2.5 mM calcium. Curves 1B, 1C and 1E are original registrations, 1A and 1D were replotted. The arrows indicate the direction of the automatic frequency change. *1A* Initial control. *1B* With 10^{-5} M orciprenaline[8] plus 3×10^{-4} M adenosine; 2 extrasystoles. *1C* With 10^{-5} M orciprenaline, adenosine increased to 10^{-3} M. *1D* Control curve after thorough wash-out of drugs. *1E* With 10^{-5} M orciprenaline alone.

Methods. The various approaches to conduct interval-strength studies[2] all seem quite tedious, e.g. repeated determination of steady-state contractile strength at a number of selected stimulation rates, from which 'frequency-force curves' can be plotted. In order to facilitate and standardize this procedure, we use a new stimulator[3] which can vary the driving frequency ν continuously as exponential function of time ($\nu \sim e^{t/k}$ and $\nu \sim e^{-t/k}$); an upper frequency limit is set for automatic reversal from rise to fall, usually at 1.5 Hz. The time constant k is set long enough (about 4 min) to allow almost complete cumulation of inotropic effects of activation[1] in the preparation. Peak developed isometric force is electronically processed for continuous registration[4]: 1. With an x–y recorder continuous frequency-force plots are obtained by directly recording the developed force on the y-axis against the changing frequency on the x-axis (figures 1 and 2, 1A–E, 2A–E). 2. Force responses to step changes of frequency are recorded on a conventional slow recorder (figure 2b, c, e).

All experiments were performed on isolated left atria of young male guinea-pigs. Krebs-Henseleit solution[5] with 15 mM glucose was rapidly recirculated past the tissue by bubbling with 5% CO_2 in O_2 in a special bath vessel[6,7]. The atria were stimulated slightly above threshold at 0.1–0.2 msec via a punctate cathode[6].

Results and discussion. On 150 preparations more than 1000 curves were determined under a variety of experimental conditions. The interaction of β-adrenergic

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