Central effect of 5,8,11,14-eicosatetraenoic acid (arachidonic acid) on the temperature in the conscious rabbit

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Summary. Hyperthermia induced by arachidonic acid in rabbit was attenuated by phenoxybenzamine, cyproheptadine and indomethacin. The reduction in arachidonic acid hyperthermia, after 6-OH-DA and the failure of PCPA to reduce this rise, indicates the involvement of noradrenaline in arachidonic acid hyperthermia.

The effect of the monoamines on body temperature vary from species to species. Prostaglandins of the E series (PGE) cause an increase in body temperature in all the mammalian species so far investigated[1-7]. It has been already reported that not only PGE_2 but also its precursor 5,8,11,14-eicosatetraenoic acid (arachidonic acid) produced dose-dependent hyperthermia when injected intracerebroventricularly (i.c.v.) in rabbits[8,9]. The present investigation was undertaken: a) to elucidate the nature of central receptors involved in arachidonic acid hyperthermia; b) to find out the interrelations between arachidonic acid and biogenic amines in the temperature regulation; and c) to verify in vivo the hypothesis proposed by Vane[10] that antipyretics and anti-inflammatory drugs inhibit the synthesis of PGs from their precursors[11,12]. This present report describes the effect of cyproheptadine, phenoxybenzamine and pimozide on the hyperthermia induced by i.c.v. administration of arachidonic acid in rabbits. In addition, the effect of indomethacin, 6-hydroxydopamine (6-OH-DA), which is a central depletor of noradrenaline, and para-chloro-phenyl-alanine (PCPA), which is a central depletor of 5HT, were investigated.

Materials and methods. Male albino rabbits weighing 2–3 kg were used. Animals whose temperature were either too high (> 39.9 °C) or too variable were discarded. Groups of at least 4 animals were used; precise numbers for most experiments are given in the legends of the figures. I.c.v. injection was made with a 'direct injection' technique described by Jacob et al.[13], in which pyrogen-free saline had practically no effect on the temperature. The volume of injection was 0.05 ml/kg.

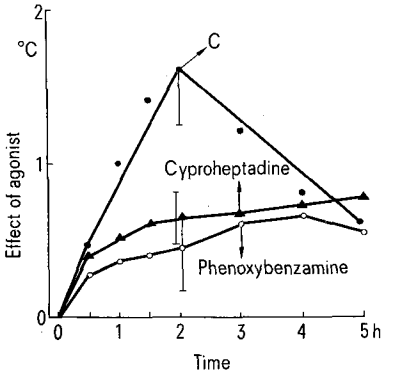

Fig. 1. Effects of phenoxybenzamine, 1 mg/kg i.v., and cyproheptadine, 3 mg/kg i.v., on hyperthermia induced by arachidonic acid (C), 100 µg/kg i.c.v. Each point shows the difference between phenoxybenzamine or cyprohepatadine control temperatures and those observed with the agonist. Abscissa: 0 = time of the i.c.v. injection of the agonist. C = effects of arachidonic acid (16 animals), experimental groups: 4 animals. Bars show SE of the mean.

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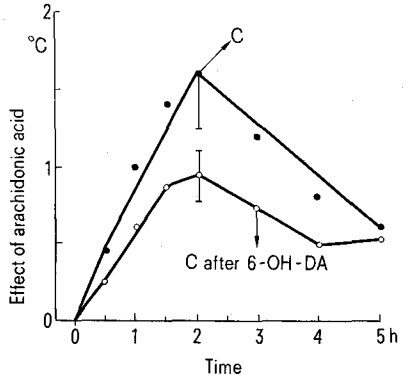

Fig. 2. Temperature response of rabbits to arachidonic acid (C), 100 µg/kg i.c.v., before and after depletion of brain noradrenaline by 6-OH-DA, 500 µg/kg i.c.v., dosed on days 1, 4 and 7.
Abscissa: 0 = time of the i.c.v. injection of arachidonic acid; C as in figure 1. Experimental group: 6 animals.

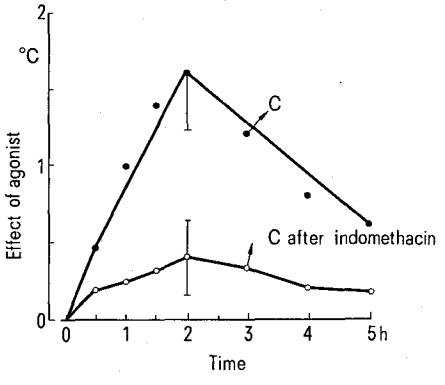

Fig. 3. Effect of indomethacin, 10 mg/kg s.c., on hyperthermia induced by i.c.v. arachidonic acid (C), 100 µg/kg. i.c.v. Points obtained as described in figure 1.
Abscissa: 0 = time of the i.c.v. injection of the agonist. C as in figure 1. Experimental group: 4 animals.

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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