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Inhibition of prostaglandin generation in the rabbit brain in-vivo by AD-1590, a non-steroidal anti-inflammatory agent with potent antipyretic activity

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The inhibition of prostaglandin generation by AD-1590 was investigated in the rabbit brain in-vivo. AD-1590 (0·4 mg kg^{-1} i.v.) markedly prevented both the increases in body temperature and PGE_2 level in cerebrospinal fluid (CSF) caused by i.v. injection of lipopolysaccharide. On the other hand, 2,4-dinitrophenol (20 mg kg^{-1} i.v.)-induced hyperthermia, which was not affected by AD-1590, was not accompanied by an increase in PGE_2 level in CSF. When injected intracerebroventricularly, AD-1590 dose-dependently inhibited the hyperthermia caused by arachidonic acid given by the same route; its ED50 was 1·6 μg compared with about 35 μg for indomethacin. From these results, it is suggested that AD-1590 is more active than indomethacin in suppressing prostaglandin synthetase in rabbit brain.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin are thought to produce their anti-inflammatory, analgesic and antipyretic actions by inhibition of prostaglandin synthesis (Flower & Vane 1974). There is a good relation between their ability to inhibit prostaglandin generation and their anti-inflammatory activity (Gryglewski 1979), and most have well-balanced anti-inflammatory, analgesic and antipyretic activities. However, AD-1590, a new acidic NSAID, has been reported to have a relatively potent antipyretic activity compared with its other actions, its antipyretic activity being more than 50 times that of indomethacin in lipopolysaccharide (LPS) febrile rabbits while its anti-inflammatory activity is comparable with that of indomethacin (Nakamura et al 1983, 1984a). LPS is generally considered to produce fever through production and release of prostaglandins, especially the E-series, within the hypothalamus although there are some findings in conflict with this view (Rosendorff & Woolf 1979; Milton 1982). Therefore, AD-1590 may be more effective in inhibiting prostaglandin synthetase in the brain than that in the peripheral tissues. We have therefore investigated the inhibitory activity of AD-1590 on prostaglandin generation in the brain in-vivo to ascertain the mode of its superior antipyretic action and also the role of prostaglandins in the pathogenesis of LPS-induced fever. The inhibitory activity of AD-1590 on in-vivo prostaglandin generation in rabbit brain was compared with that of indomethacin.

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MATERIALS AND METHODS

Implantation of guide tube

Male, albino rabbits, 2·3–3 kg, were used. Under sodium pentobarbitone (30 mg kg^{-1} i.v.) anaesthesia, a cannula (o.d. = 0·7 mm) was implanted in the brain at the level of the right lateral cerebral ventricle; the coordinates according to the brain atlas of Fifková & Marsála (1967) were: −1 mm anterior, +2·3 mm lateral, 9 mm high. Animals were allowed at least one week to recover.

Antipyretic assay

Pyrexia was induced by an intravenous injection of 1 μg kg^{-1} of LPS (lipopolysaccharide B., *E. coli* 026:B_6, Difco) dissolved in sterile 0·9% NaCl (saline). Hyperthermia was induced by an intracerebroventricular (i.c.v.) injection of 100 μg (in 20 μl saline) of arachidonic acid sodium salt (Sigma) or by an intravenous injection of 20 mg kg^{-1} of 2,4-dinitrophenol (DNP, Wako Chem. Ind.) dissolved in saline.

The rectal temperature was monitored by means of a thermistor probe (Takara Thermistor K-700, Takara Ind., Japan), and recorded automatically at 5 min intervals for 2 h before and 3–5 h after injec-

tion of the pyrogenic substance. The fever index refers to the area under the fever curve for 3 h after the arachidonic acid injection; one unit of the fever index is equivalent to a 1 °C change lasting for 1 h (Clark & Cumby 1975).

Drugs were administered intravenously just before LPS or DNP, or intracerebroventricularly 15 min before arachidonic acid.

PGE_2 measurement

The cerebrospinal fluid (CSF, 20 or 40 µl) taken through the guide tube was poured into an ice-cold test tube containing 1 or 0·2 ml of the assay buffer from the radioimmunoassay kit and the tube was frozen until use.

A PGE_2 radioimmunoassay kit (New England Nuclear, USA), based on the use of an ^{125}I-analogue of PGE_2 as tracer and rabbit anti-PGE_2 as specific antibody, was used. Due to the high concentration of this prostaglandin in the cerebrospinal fluid of rabbits, it was determined without any previous extraction procedure. In our procedure the sensitivity was approximately 40 pg ml^{-1} CSF.

Drugs

2-[8-Methyl-10,11-dihydro-11-oxodibenz[b,f]-oxepin-2-yl]propionic acid (AD-1590) was dissolved in distilled water just before use with an appropriate quantity of 0·1 M NaOH; indomethacin was dissolved in 0·1 M phosphate buffer (pH 7·4).

Statistical analysis

Student's *t*-test was used for the statistical analyses. The ED50 value was calculated from the effective rates according to Litchfield & Wilcoxon (1949).

RESULTS

Blockade of LPS-induced increase of PGE_2 level in CSF by AD-1590

The PGE_2 level in the CSF was measured simultaneously with body temperature after LPS injection. After LPS injection, the mean PGE_2 level of the control was increased about 6 times the base line value and there was a good relation between the mean increases in body temperature and PGE_2 level (Fig. 1). However, a precise relation between increases in individual animals was not found, since the level varied between individuals. AD-1590 (0·4 mg kg^{-1}) produced a marked reduction in the increase in body temperature and PGE_2 level after an intravenous injection.

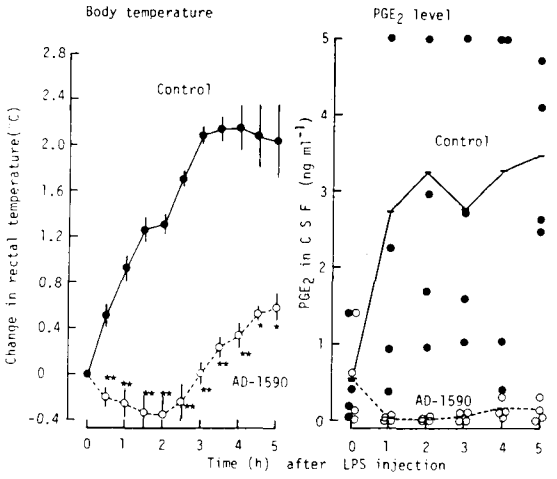

FIG. 1. Effect in rabbits of AD-1590 on fever and PGE_2 level increase induced by LPS in CSF. AD-1590 (0·4 mg kg^{-1}) was administered just before LPS (1 µg kg^{-1} i.v.) injection. Body temperature: each point and vertical bar represent the mean and s.e.m. from 4 rabbits; $*0{\cdot}01<P<0{\cdot}05$ & $**P<0{\cdot}01$ significantly different from each control. PGE_2 level is presented both by the individual (point) and mean (line) value (control: closed circle & solid line; AD-1590: open circle & dotted line); results more than 5 ng ml^{-1} are marked at 5 ng ml^{-1}.

PGE_2 level in CSF in DNP-induced hyperthermia

DNP produced a marked, dose-related hyperthermia at doses of 10 to 30 mg kg^{-1} i.m. in rabbits; the maximal increases in rectal temperature were 0·244 (n =5), 1·20 (n = 4) and 2·43 (n = 3) °C at 10, 20 and 30 mg kg^{-1}, respectively. Then DNP was also administered intravenously at 20 mg kg^{-1}.

After DNP 20 mg kg^{-1} i.m., there was a marked hyperthermia but no increase in PGE_2 level in the CSF (Fig. 2). AD-1590 (0·4 mg kg^{-1}) reduced the PGE_2 content to below the control level 1 and 3 h after DNP injection, but failed to inhibit the hyperthermia at an intravenous dose

Inhibitory activity of AD-1590 against hyperthermia induced by arachidonic acid

Arachidonic acid produced a marked, lasting rise in rectal temperature after 100 µg i.c.v. (as sodium salt); the mean rises and s.e.m. from 7 rabbits were 0·93 ± 0·15, 1·77 ± 0·21 and 1·81 ± 0·19 °C at 1, 3 and 5 h post injection, respectively; the mean and s.e.m. of the fever index (0–3 h) was 3·50 ± 0·48.

AD-1590, when injected intracerebroventricularly 15 min before arachidonic acid, markedly inhibited the hyperthermia at doses of 1·6 to 12·8 µg (Fig. 3). Indomethacin also showed a significant inhibitory

activity at 51·2 μg i.c.v. The number of animals per group with a fever index of 2 or less was 3/6, 5/7, 3/3 and 4/4 at doses of AD-1590 of 1·6, 3·2, 6·4 and 12·8 μg i.c.v., respectively, and 1/5, 1/3 and 2/4 at

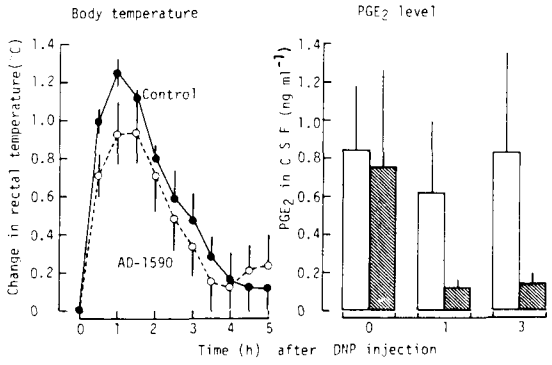

FIG. 2. Effect in rabbits of AD-1590 on body temperature and PGE_2 level in CSF after DNP injection. AD-1590 (0·4 mg kg^{-1} i.v.) was administered just before DNP (20 mg kg^{-1} i.v.) injection. Vertical bars represent s.e.m. (n = 4). Body temperature and PGE_2 content were measured independently. Open column: control. Shaded column: AD-1590.

doses of indomethacin of 12·8, 25·6 and 51·2 μg i.c.v., respectively. Thus, the ED50 of AD-1590 and indomethacin was 1·6 and about 35 μg i.c.v., respectively.

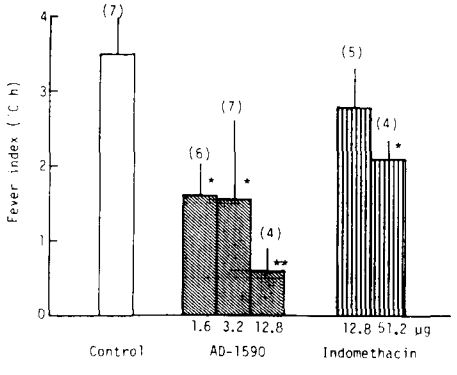

FIG. 3. Inhibitory activity in rabbits of AD-1590 and indomethacin administered i.c.v. on hyperthermia induced by arachidonic acid. Drugs were administered i.c.v. 15 min before arachidonic acid (100 μg i.c.v. as sodium salt). Each column and vertical bar represent the mean fever index (0–3 h) and s.e.m. ( ) number of rabbits used. *0·01<P<0·05 & **P<0·01 significantly different from the control.

Inhibitory activity of AD-1590 and indomethacin on arachidonic acid-induced increase in the PGE_2 level in CSF

The PGE_2 level in CSF was increased about 13 and 10 times at 1 and 3 h, respectively, after an i.c.v. injection of arachidonic acid. Pretreatment with 1·6 μg of AD-1590 i.c.v., which produced a significant inhibitory activity against arachidonic acid-induced hyperthermia, resulted in a significant decrease in PGE_2 level (Fig. 4). This dose of AD-1590 was comparable to that of 35 μg i.c.v. of indomethacin.

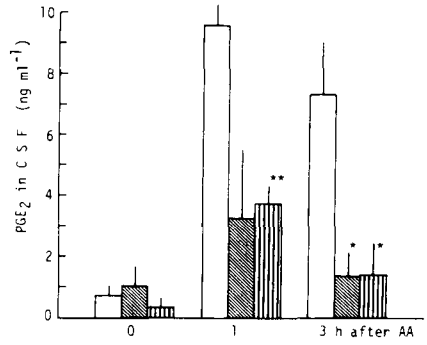

FIG. 4. Effect in rabbits of AD-1590 and indomethacin on arachidonic acid-induced increase in PGE_2 level in the CSF. AD-1590 (1·6 μg i.c.v.; shaded column) and indomethacin (35 μg i.c.v.; striped column) were injected 15 min before arachidonic acid (AA) i.c.v. Each column and bar show the mean and s.e.m. from 3 rabbits (PGE_2 content more than 10·25 ng ml^{-1} was considered as 10·25 ng ml^{-1}). *0·01<P<0·05 & **P<0·01 significantly different from the control (open column).

DISCUSSION

Endogenous pyrogen, produced and released by leucocytes, is thought to enter the central nervous system and act within the hypothalamus to produce fever through production and release of prostaglandins, while NSAIDs produce their antipyretic activity by inhibiting prostaglandin generation within the hypothalamus (Clark 1979). We have previously found that AD-1590, like indomethacin, produces its antipyretic action through inhibition of prostaglandin synthesis within the hypothalamus (Nakamura et al 1984b). This finding, however, is not enough to explain the potent antipyretic activity of AD-1590.

A high level of E-series prostaglandins in CSF has been found in febrile animals and man (Philipp-Dormston & Siegert 1975; Milton 1982). AD-1590 prevented both the increases in body temperature

and PGE_2 level in the CSF induced by LPS. On the other hand, DNP-induced hyperthermia, which was not affected by AD-1590, was not accompanied by an increase in PGE_2 level in the CSF. These results indicate that AD-1590 selectively blocks the increase in body temperature caused by an enhancement of brain prostaglandin generation.

Arachidonic acid causes a lasting hyperthermia when injected intracerebroventricularly in rats, cats or rabbits over a dose range of 10 to 100 μg per animal (Splawiński et al 1974; Clark & Cumby 1976, 1981; Laburn et al 1977; Hertz et al 1979; Townsend et al 1981); NSAIDs prevent this hyperthermia when given systemically, but there is no data on the effects of i.c.v. injections of NSAIDs. When injected i.c.v. at a dose of 1·6 μg, about 100 times lower than its antipyretic ED50 (0·08 mg kg^{-1} i.v.) (Nakamura et al 1984a), AD-1590 suppressed both the increases in body temperature and the PGE_2 level in the CSF caused by i.c.v. injection of arachidonic acid; its potency was about 20 times that of indomethacin. This indicates that AD-1590 strongly inhibits prostaglandin generation within the brain.

AD-1590 is about 2·7 times more potent than indomethacin in inhibiting prostaglandin generation in-vitro by rabbit renal microsomes (Nakamura et al 1983). This potency ratio of AD-1590 to indomethacin roughly agrees with the in-vivo potency ratios in anti-inflammatory and analgesic action; AD-1590 is about 2·3 and 1·8 times that of indomethacin in carrageenan-induced hind paw oedema and acetic acid-induced writhing in rats. However, its antipyretic activity is more than 50 times that of indomethacin in LPS-febrile rabbits. Therefore, it may be possible that AD-1590 has a greater central than peripheral activity similar to that of paracetamol (Flower & Vane 1972) and Dipyrone (Dembińska-Kieć et al 1976).

There is a good relation between inhibition of prostaglandin synthesis and anti-inflammatory activity of NSAIDs (Flower & Vane 1974; Gryglewski 1979). However, the generation of prostaglandin from added arachidonic acid by microsomal fractions and its inhibition by NSAIDs varies with the tissue used. There is evidence to show that inhibition of prostaglandin synthesis by NSAIDs varies between tissues. Indomethacin inhibits activity in rabbit spleen > kidney > brain (Dembińska-Kieć et al 1976) and is 21 times more potent in dog spleen than in rabbit brain (Flower & Vane 1972). Aspirin is more effective in suppressing the release of $PGF_{2\alpha}$ from platelets than from synovium in man (Patrono et al 1976). Flufenamic acid and azapropazone reduce prostaglandin levels in pig plasma without affecting levels in the gastric mucosa (Rainsford et al 1981) and there are considerable differences in the sensitivity of various vascular beds in the rabbit to NSAIDs (Hadházy et al 1984). Prostaglandin synthetase is a membrane-bound, haem-containing protein, and the two distinct activities, oxygenation and peroxidation are present in a single polypeptide chain (Roth et al 1980). NSAIDs interact with at least two distinct sites on prostaglandin synthetase (cyclo-oxygenase), a catalytic site and a supplementary one with which indomethacin and aspirin, respectively, interact more effectively (Humes et al 1981; Rotilio et al 1984). There is the possibility that some NSAIDs interact with the synthetase (or its isozyme) and/or gain access to the enzyme in a mode somewhat different from other NSAIDs. AD-1590, which has a large, comparatively rigid alicyclic moiety may have some selectivity for the brain enzyme.

The results demonstrate that the large difference in antipyretic activity between AD-1590 and indomethacin appears to be attributable to the fact that AD-1590 is more selective for the prostaglandin synthetase in the brain than is indomethacin.

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