A comparison of anti-inflammatory and other compounds on the spontaneously contracting pregnant rat uterus

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Recently Lewis, Cottney & Sugrue (1975) suggested that the uterus of the 17–21 day pregnant rat might provide a suitable model for the detection of anti-inflammatory drugs. The use of this tissue was based upon earlier experiments showing that locally produced prostaglandins are the mediators of the spontaneous contractions in the pregnant rat uterus (Aiken 1972; Vane & Williams 1973) and that with the exception of steroidal agents there was a good correlation between inhibition of prostaglandin synthetase assayed *in vitro*, inhibition of uterine contractions and inhibition of carageenan-induced rat paw oedema.

In our laboratory, crude extracts from a variety of marine organisms are routinely screened for biological activity. Isolated organ techniques offer definite advantages for such screening, principally due to simplicity and the small amounts of material needed for testing. The isolated uterus provides a much simpler method than either the biochemical assays for screening compounds for inhibition of prostaglandin synthetase activity, or the rat paw oedema techniques. We have therefore studied this preparation as a possible screening method for new anti-inflammatory compounds, and feel that several conclusions about the sensitivity and specificity of the preparation for this purpose should be noted.

Uteri from 17–21 day pregnant Fullinsdorf rats were removed, slit longitudinally, and the contents discarded. A 4–5 cm length of uterine tissue, proximal to and including the ovary, was suspended in Kreb's bicarbonate solution at 37°, and gassed with 5% CO_2 in oxygen. Spontaneous contractions were recorded through an isotonic transducer against a load of 1·5 g. The uteri were allowed 30 min to equilibrate, washed, and then left until regular, spontaneous contractions were exhibited. These contractions continued for up to 8 h in our experiments.

The sensitivity of our preparations of the isolated uterus to anti-inflammatory agents was ten to a hundred times lower than that reported by Lewis & others, 1975 (Table 1), while being compatible with that reported earlier by Vane & Williams (1973). Aiken (1972) reported that the preparation was more sensitive to anti-inflammatory agents when they were added immediately after washing, i.e. while the concentration of prostaglandins in the organ bath was low. However, in our experiments the time taken for the return of regular full-scale spontaneous activity after washing the isolated uterus varied from 5 to 30 min, making comparisons of inhibitory activity inaccurate during this post-wash period.

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Our studies with known non-steroidal anti-inflammatory compounds qualitatively agreed with the results of Lewis & others (1975) in that there was a correlation between the effect of these compounds on the isolated rat uterus, prostaglandin synthetase and rat paw oedema. However, no such correlation existed for a variety of other substances which we found to be potent inhibitors of the pregnant rat uterus preparation. Such compounds included:

(i) the smooth muscle relaxant papaverine which is known not to effect prostaglandin production in the uterus (Aiken 1972); (ii) an anaesthetic, ketamine, which inhibits drug-induced contractions of the guinea-pig ileum and relaxes other smooth muscle preparations (Lundy, Gowdey & Colhoun, 1974); (iii) isoprenaline and other β-adrenoceptor agonists; (iv) the cns active compounds chlorpromazine, desipramine and diazepam which are known to inhibit the release of prostaglandins by the uterus (Tothill, Bamford & Draper, 1971, Kunze, Bohn & Bahrke, 1975); (v) the local anaesthetic procaine which has been shown to inhibit prostaglandin synthesis in beef and sheep seminal vesicles (Kunze Bohn & Vogt, 1974).

Yet none of these compounds (with the exception of chlorpromazine) had any anti-inflammatory activity as indicated by their ability to prevent carageenan-induced oedema in the rat paw (Table 1).

The initial effect of low concentrations of papaverine, ketamine and isoprenaline was a reduction in force but an increase in rate of contraction of the isolated preparation (Fig. 1). In contrast, chlorpromazine,

Table 1. *A comparison of activities of compounds on the spontaneously contracting pregnant uterus and carageenan-induced rat paw oedema.*

Compound	Concn for 50 % inhibition of force of contraction of uterus (g m^{-1})	Effect on frequency of contractions	Oral dose for 50 % inhibition of carageenan-induced rat paw oedema (mg kg^{-1})*
Indomethacin	1×10^{-6}	Decreased	3
Phenylbutazone	5×10^{-6}	Decreased	30
Acetylsalicylic acid	1×10^{-4}	Decreased	150
Flufenamic acid	1×10^{-5}	Decreased	10
Isoprenaline	5×10^{-10}	Increased	Inactive
Procaine	$>4 \times 10^{-3}$	Increased	Inactive
+Papaverine	1×10^{-5}	Increased	Inactive
+Ketamine	1×10^{-4}	Increased	Inactive
+Diazepam	3×10^{-5}	Unchanged	Inactive
+Chlorpromazine‡	2×10^{-6}	Unchanged	10
+Desipramine	5×10^{-6}	Unchanged	Inactive

+ Dose of 10 mg kg^{-1} in rat paw oedema test.
* Measured as a percentage inhibition of the oedema induced by intradermal injection of 0·5 mg of carageenan in 0·9 % saline into the plantar suface of the rat hind paw.
‡ 23% inhibition only.

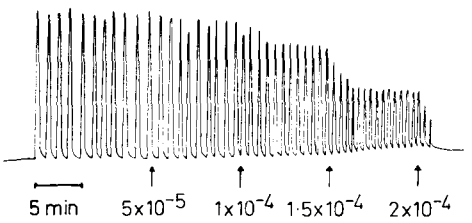

FIG. 1. The effect of ketamine g ml^{-1} on the contractions of the pregnant rat uterus. Note that the rate of contractions increases with the lower concentrations of this agent.

desipramine and diazepam all decreased the force without affecting the rate of contraction. However, the potent anti-inflammatory agents indomethacin, phenylbutazone, acetylsalicylic acid and flufenamic acid reduced both rate and force of contraction of the isolated uterus preparation. Contractions of the uterus were completely inhibited by higher doses of all of the above compounds.

Our studies suggest that although the isolated pregnant rat uterus is a simple technique for detecting compounds that inhibit prostaglandin synthetase activity, the results must be interpreted with caution due to the lack of specificity of the preparation. The use of the preparation as a quantitative technique is also limited as some inhibitors of prostaglandin synthetase such as indomethacin have, in addition, inhibitory effects on smooth muscle by mechanisms independent of any effect involving prostaglandin synthesis (Northover, 1971; Flores & Sharp, 1972).

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Anti-inflammatory action of azapropazone

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Azapropazone (Rheumox, A. H. Robins Ltd.) is recommended in the treatment of rheumatoid disease at a dose of 1200 mg day^{-1}. In previous publications (Lewis, Capstick & Ancill, 1971; 1972), we have shown that the drug possesses some biochemical properties in common with some other non-steroidal antirheumatic agents which, theoretically at least, could form a basis for their anti-inflammatory action.

The anti-inflammatory action of azapropazone has been demonstrated on acute inflammatory animal models, such as bradykinin-induced inflammation, ultraviolet erythema and the rat paw oedema tests (Jahn & Wagner-Jauregg, 1974).

We now report on the action of azapropazone on the development of granulation tissue in cotton wool pellets implanted in the rat and on the polyarthritis in the adjuvant arthritic rat. We have also re-examined the action of azapropazone on membranes.

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Our previous model involving rat liver lysosomes is subject to the disadvantages pointed out by Ignarro (1971) in that different experimental conditions can lead to different results. In this work we have examined the action of azapropazone on polymorphonuclear leucocytes (polymorphs) since these cells are considered to make a sizeable contribution to the release of lysosomal degradative enzymes in the joints of patients with rheumatoid disease (Smith & Hamerman, 1962). Adjuvant arthritis was induced in Wistar strain male rats (0·2 kg) as previously described (Lowe, 1964). Treated rats were dosed daily by intraperitoneal injection with either 20 or 40 mg kg^{-1} of azapropazone dispersed in saline by compound tragacanth powder B.P. The azapropazone was omitted from the controls. Primary and secondary inflammation was assessed by measuring foot volume by means of a mercury bath connected with a pressure transducer linked to a Devices recorder. The volumes of both hind feet were measured.