The spontaneously contracting pregnant rat uterus as a model for anti-inflammatory drug activity

One of the proposed mechanisms of action of anti-inflammatory drugs is the inhibition of prostaglandin synthetase. The correlation between anti-inflammatory activity and prostaglandin synthetase inhibition has been demonstrated using purified enzyme from a variety of tissues (for references see Ferreira & Vane, 1974). The possibility of using a further *in vitro* indicator of prostaglandin synthetase inhibition was suggested by the discovery that uteri of pregnant rats exhibited spontaneous contractions which were a result of prostaglandin release (Vane & Williams, 1973). Since the anti-inflammatory drugs indomethacin and meclofenamate inhibited this spontaneous activity it was of interest to investigate the effect of other anti-inflammatory agents on this preparation and establish whether such activity could be correlated with their inhibitory effects on prostaglandin synthetase enzyme preparations described by other workers. No comprehensive data are available on the uterine PG synthetase drug sensitivity in the rat and so data described for the microsomal fraction of dog spleen has been used for this purpose (Vane, 1972). Furthermore the anti-inflammatory potency of these drugs, as determined by kaolin-induced rat paw oedema, has been assessed.

Intact uterine horns from female Wistar rats (180–200 g), 17–22 days pregnant, were mounted in Krebs solution at 37° and bubbled with 5% CO_2 in oxygen. Spontaneous contractions were recorded isotonically against a load of 1·5 g. Drugs were administered after a 25 min stabilization period and the effects were examined over a 25 min incubation period before washout. Mean contraction heights and mean frequencies of contraction for the same duration (15 min) before and after drug were measured and drug induced inhibitory effects on both parameters were measured, pooled and the mean percentage inhibitory response was calculated for each drug concentration. ID50 values were subsequently calculated from the resulting dose response curve.

Regular spontaneous contractions occurred for as long as 2 h in control uteri although not all uteri demonstrated such regular activity (there was little variation between preparations over 4 months of testing). Results for uteri with irregular activity (5% of total examined) were discarded. All drugs examined inhibited the spontaneous contractions of the uterine horn. Drug onset time varied only slightly and effects were maximal within 10 min. Washout of the low concentrations of drug resulted in return of activity in most instances (90% of the preparations examined) allowing dose response relationships to be calculated.

The rank order of potency of these drugs using these ID50 data was somewhat different from that described for inhibition of the dog spleen microsomal fraction containing PG synthetase by Vane (1972) (Table 1). However, it should be noted that the potency of drugs inhibiting prostaglandin synthetases from different sources does vary (Flower, 1974).

There is, with certain exceptions, a correlation between the ability of the drugs to inhibit contractions of the uterus and their potency as both anti-inflammatory agents and prostaglandin synthetase inhibitors. However, naproxen exhibits more potent anti-inflammatory and prostaglandin synthetase inhibition (Ham, Cirillo & others, 1972) than either phenylbutazone, ibuprofen or aspirin yet is less active on the spontaneously contracting uterus. Paracetamol, which possesses minimal anti-inflammatory activity and little systemic prostaglandin synthetase inhibitory activity is also shown to be a more effective blocker of the spontaneously contracting uterus than oxyphenbutazone. The steroidal agents tested are both relatively ineffective in inhibiting uterine contractions and also in inhibiting prostaglandin synthetase *in vitro*.

Table 1 *A comparison between drug activities measured using the spontaneously contracting rat uterus, an* in vitro *enzyme assay of prostaglandin synthetase activity and kaolin-induced rat paw oedema.*

Drug	Drug induced inhibition of rat uterus ID50 (μg ml^{-1})	Drug induced inhibition of prostaglandin synthetase ID50[a] (μg ml^{-1})	Anti-inflammatory activity ID50[b] (mg kg^{-1})
Indomethacin	0·05	0·06	4·8
Phenylbutazone	0·5	2·23	58
Ibuprofen	0·61	NE[c]	165
Aspirin	1·0	6·61	190
Naproxen	7·5	NE	4·7
Paracetamol	15·0	100	>300
Oxyphenbutazone	25·0	NE	105
Dexamethasone	350·0	10% inhibition at 100 μg ml^{-1}	0·34
Hydrocortisone	1500·0	3% inhibition at 100 μg ml^{-1}	67
S.C. 19220	2·0	NE	>50 (if any)

[a] Data taken from Vane (1972) using the microsomal fraction of dog spleen as the source of prostaglandin synthetase.

[b] Data obtained from rat paw oedema tests using kaolin (0·1 ml 10% administered via the subplantar route) as irritant. The drugs were administered subcutaneously in 5% mulgofen 60 min before the kaolin. [c] Not examined.

The prostaglandin antagonist, S.C. 19220 [1-acetyl-2-(8-chloro-10,11-dihydrodibenz-(b,f) (1-4)oxazepine-10-carbonyl) hydrazine], is a good antagonist of the contractile response yet does not demonstrate marked anti-inflammatory activity in the kaolin-induced rat paw oedema.

Although all the anti-inflammatory drugs tested demonstrate inhibition of the spontaneous activity of the pregnant uterus it is not certain how they are exerting this effect. It is reasonable to assume that PG synthetase inhibition occurs although it is conceivable that higher concentrations of the drugs may inhibit the responses to the prostaglandins released by the contracting uterus in a similar fashion to S.C. 19220. This explanation is unlikely, however, since studies examining possible prostaglandin antagonism by several non-steroidal anti-inflammatory drugs have failed to demonstrate antagonism of prostaglandin responses on the rat stomach strip and rat colon at doses producing up to 75% inhibitory responses in the present experiment (unpublished observations). Some of the drugs may complex with calcium ions in the incubation fluid and this could also result in inhibition of contractile responses.

From these data it appears that inhibition of the spontaneously contracting rat uterus may indicate *in vitro* prostaglandin synthetase inhibitory activity and/or prostaglandin antagonism and also allow assessment of potency of anti-inflammatory activity.

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