Metyrapone inhibits prostaglandin synthesis and release from the pregnant rat uterus *in vitro*

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Metyrapone, an inhibitor of corticosteroid biosynthesis (Chart & Sheppard, 1959), causes foetal adrenal hypertrophy (Dupouy, 1972) and prolongs pregnancy in the rat (Parvez, Parvez & Roffi, 1972). As the foetuses exert some form of control over prostaglandin (PG) release from the pregnant rat uterus *in vitro* (Parnham, Sneddon & Williams, 1975), the effect of metyrapone on this uterine PG release has been investigated.

Two experimental approaches were used. First, pregnant rats received daily injections of either metyrapone (150 mg kg^{-1}, s.c. or i.p.) or vehicle (0.33 M (+) tartaric acid, 0.75 ml kg^{-1}, s.c. or i.p.) on days 20 and 21 of pregnancy. The animals were then killed on day 22 and the PG release from single uterine horns determined by the method of Vane & Williams (1973). Secondly, PG synthetase activity in day 22 pregnant uterine homogenates was determined by incubation for 10 min with ^{14}C-arachidonic acid and measuring its conversion to PGE_2 and $PGF_{2\alpha}$ which were separated on silicic acid mini-columns. Incubations were carried out in the presence of metyrapone (0.005-0.05 mM) against boiled blanks and buffer controls.

Metyrapone significantly reduced uterine PG release *in vitro* from 253 ± 35.5 (s.e. mean) ng g^{-1} h^{-1} in control uteri to 174 ± 21.2 ng g^{-1} h^{-1} in metyrapone-treated uteri ($P < 0.05$). The results of Parvez *et al.* (1972) showing prolongation of pregnancy by metyrapone (150 ng kg^{-1}) were also confirmed in a group of 13 rats. Metyrapone showed a dose-dependent inhibition of PGE_2 synthesis *in vitro* as determined by the conversion of ^{14}C-arachidonic acid to PGs. $PGF_{2\alpha}$ synthesis showed an apparent stimulation with metyrapone, probably due to redirection of synthesis from PGE_2. Preliminary experiments with indomethacin have confirmed that this inhibitor blocks the synthesis of both PGE_2 and $PGF_{2\alpha}$.

These results suggest that metyrapone is a selective inhibitor of uterine PGE_2 synthesis and this may account for the prolongation of pregnancy by metyrapone in the rat.

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Action of spasmogenic substances on Ca^{2+} movements of rat uterine smooth muscle

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These experiments were performed on uteri obtained from virgin Wistar rats of 180-200 g weight, pretreated the day before the experiment with 0.5 mg/kg of diethylstilboestrol diproprionate. Myometrium strips were prepared by excising the mucous and submucous layers.

The role of extracellular Ca^{2+} in myometrial contractility was examined on strips mounted isotonically. In at least five experiments angiotensin II (At II) 2.5×10^{-6} M, serotonin (5-HT) 2.5×10^{-5} M and carbachol (CCh) 2.5×10^{-5} M, produce a maximum contraction in a normal Ringer solution. In each experiment the contraction produced was less than 10% of the control as soon as 3 min after exposure of the strips to a solution without Ca^{2+}. The contractile response was restored to control levels less than 3 min after readmitting Ca^{2+} 1.5 mM to the bath. These results suggest that: (1) activation of contraction is due to an increase in membrane

permeability to Ca^{2+}, or (2) extracellular Ca^{2+} is necessary to maintain intracellular Ca^{2+} stores at a normal level. In order to examine these possibilities, we have studied the action of the spasmogenic substances on ^{45}Ca uptake and efflux. ^{45}Ca uptake was determined with the La^{3+} technique (Mayer, Van Breemen & Casteels, 1972). At II 2.5×10^{-6} M, and CCh 2.5×10^{-5} M did not produce any significant change in uptake curves. On the other hand, KCl 101 mM increased ^{45}Ca uptake at 5, 10, 20 and 30 min exposure to the radioactive solution as well as the exchangeable Ca^{2+} that increased from the control value of 0.163 ± 0.007 mM/kg to 0.244 ± 0.013 mM/kg (10), $P < 0.02$. 5-HT 2.5×10^{-5} M also augmented Ca^{2+} uptake at 5, 10, 20 and 30 min, as well as the exchangeable Ca^{2+} which increased from 0.168 ± 0.009 mM/kg to 0.200 ± 0.008 (14), $P < 0.01$

Efflux curves were performed after previous equilibration with the ^{45}Ca Ringer solution. The ^{45}Ca efflux was obscured by the Ca^{2+} exchange with the binding sites in the extracellular space. In order to dissociate this latter exchange from the efflux from the cellular compartment, we displaced the Ca^{2+} bound to the extracellular and superficial membrane sites by adding 20 mM Ca^{2+} ethylene glycol-bis (β-amino-ethyl ether) N,N'-tetraacetic acid (EGTA) (Van Breemen & Casteels, 1974). Under these conditions AtII 2.5×10^{-6} M did not modify the rate of efflux of rat myometrium which varied from 13.7 ± 1.3 to 15.36 ± 0.6 $min^{-1} \times 10^{-3}$ (8), $P < 0.20$. On the contrary, efflux was augmented by 5-HT 2.5×10^{-5} M from 10.52 ± 0.6 to 16.27 ± 1.5 $min^{-1} \times 10^{-3}$ (4), $P < 0.01$; by CCh 2.5×10^{-5} M from 11.5 ± 0.9 to 14.25 ± 0.5 $min^{-1} \times 10^{-3}$ (4), $P < 0.01$ and by KCl 101 mM from 10.20 ± 0.1 to 13.70 ± 0.1 (4), $P < 0.05$. These increases of efflux may be due to a greater membrane permeability to Ca^{2+} or to the release of Ca^{2+} from intracellular binding sites.

In conclusion, AtII and CCh activate uterine smooth muscle contraction by a displacement of Ca^{2+} from intracellular stores, since Ca^{2+} uptake was not affected. The Ca^{2+} stores were in equilibrium with extracellular Ca^{2+}. On the other hand, KCl and 5-HT act at least in part by increasing the membrane permeability to Ca^{2+}.

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A comparison of some smooth-muscle effects of GABA and of prostaglandin E_1

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The effects of GABA on intestinal smooth muscle preparations are variable, one of the most regular being inhibition of the ACh- or nicotine-induced contractions of the guinea-pig ileum (Hobbiger, 1958; Florey & McLennan, 1959). We have found direct excitatory effects from high concentrations of GABA on the guinea-pig uterus or caecal taenia. These effects may perhaps be more closely related to the depolarizing action of GABA on some nerve cells than to its hyperpolarizing action on others. The uterus and taenia also contract in response to PGE_1 in much lower concentrations; but the longitudinal muscle of the ileum or colon is less sensitive to either spasmogen.

The concentrations of GABA needed to cause contraction are generally within the range 1-30 mmol/l, but potentiation of other spasmogens has been seen in some experiments on the uterus with 0.15 mmol/l. In some experiments, the time-course of this potentiation has resembled the prolonged 'enhancement' following brief application of PGE_1 (Clegg, Hall & Pickles, 1966). At the higher concentrations, the metabolism of GABA might provide a significant amount of energy. However, the GABA-transaminase inhibitor amino-oxyacetic acid (10-45 μg/ml) neither consistently increased nor decreased the uterine responses.

Atropine (10 μg/ml) did not alter the responses of either tissue to GABA, and neither bicuculline nor its methochloride (10-25 μg/ml) clearly inhibited the responses of the uterus. Those to PGE_1, and to GABA in a few experiments, were partially inhibited by the prostaglandin-antagonist

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