to those produced by estrogen. Compound **I** increased the uterine ratio reliably at 50 mg/kg and 250 mg/kg, but not at 10 mg/kg (table). The effects of **I** were much weaker than those of DES as is also true of 4-[2(hydroxyphenyl)-ethyl]phenol[10]. No lethality or overt toxic effects were seen in any group, but there was a loss of body weight during each experiment among the animals dosed with DES. In other testing in our laboratory, **I** has been given i.p. in male mice at doses up to 400 mg/kg without producing lethality or other observable toxicity.

The effects of compound **I** alone are not sufficient to account for the estrogenic properties attributed to *Cannabis*[4-6]. Preliminary testing with cannabispiran (**II**) revealed no significant increase in the uterine ratio at 10 mg/kg. Similar testing with dehydrocannabispiran (**III**) and β-cannabispiranol (**IV**) at 1 mg/kg also showed no significant uterine ratio increase. The increased estrogenic effect of compound **I** suggests that both cannabinoids and non-cannabinoids may be necessary to account for the estrogenic effects which are reported to result from the use of *Cannabis*.

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2 To whom correspondence should be addressed.
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Etamsylate as inhibitor of prostaglandin biosynthesis in pregnant human myometrium in vitro

L. Kovács and G. Falkay

Department of Obstetrics and Gynecology, University Medical School, Szeged (Hungary), 22 December 1980

Summary. The effects of etamsylate on prostaglandin (PG) biosynthesis in microsomes of pregnant human myometrium in vitro have been determined, and compared with those of indomethacin. Both drugs inhibited PG biosynthesis, indomethacin being the more potent inhibitor of the two. Etamsylate inhibited synthesis of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, and thromboxane B_2; increasing the concentration of etamsylate increased the inhibition of synthesis. It is suggested that etamsylate has no anti-cyclo-oxygenase activity, but acts by inhibiting the activity of prostacyclin synthetase, endoperoxide reductase, endoperoxide isomerase, and thromboxane synthetase.

Etamsylate (diethylammonium 1,4-dihydroxy-3-benzenesulfonate) is a hemostatic drug that has been shown to be effective in reducing menstrual blood loss in intrauterine-device menorrhagia[1,2], and to inhibit prostaglandin (PG) biosynthesis in the rabbit iris-ciliary body and kidney medulla[3]. PGs are implicated in the mechanism of parturition in many animal species, including man[4], and there is increasing evidence that they may play an important part in the onset and progression of labor[5,6]. That several drugs can influence the biosynthesis of PGs in the pregnant human myometrium has been demonstrated in this laboratory[7-9].

It is only recently that human myometrial tissue has been shown to synthesize prostacyclin (PGI_2) and thromboxane A_2 (TXA_2), substances that are important in the regulation of vascular tone and thereby in the regulation of blood pressure. PGI_2 and TXA_2 are unstable; they break down spontaneously and quantitatively to 6-oxo-$PGF_{1\alpha}$ and TXB_2[10]. These findings prompted this preliminary study of the effect of etamsylate on PG biosynthesis in the pregnant human myometrium in vitro. The effect of etamsylate was compared with that of indomethacin, a commonly used inhibitor of PG biosynthesis.

Materials and methods. Etamsylate (Dicynone®) was obtained from OM Laboratories, Meyrin/Geneva, Switzerland. Myometrial samples from pregnant women were obtained by excision from the edge of the surgical incision in lower uterine segment caesarean sections. Details of the procedure and of assay of PG-synthetase activity for application to human myometrial tissue have been published[7]. Briefly, the myometrial strips were homogenized with 0.1 M Tris HCl buffer (pH 7.8) and the microsomes separated by ultracentrifugation. The assay mixture (2.0 ml) contained 0.1 M Tris HCl (pH 7.8), 2.0 mM L-adrenaline, 2.0 mM glutathione, [1-^{14}C] arachidonic acid (0.1 μCi; 10 μM), different concentrations of etamsylate (0.1, 1.0, 5.0, 10.0, 100.0 mM) and of indomethacin (Chinoin Pharmaceutical Ltd.,; 0.1, 1.0, 5.0, 10.0 μM) and 2.5-3.5 mg of microsomal protein. After incubation at 37 °C

Inhibition by etamsylate and indomethacin of prostaglandin synthesis in microsomes of pregnant human myometrium

PG products	Etamsylate I_{50} (mM)	Indomethacin I_{50} (μM)
6-oxo-$PGF_{1\alpha}$	2.88	0.121
$PGF_{2\alpha}$	0.50	0.131
PGE_2+TXB_2	5.44	0.144

PG synthesis was measured as described in the text. I_{50} = concentration (μM or mM in final dilution) producing 50% inhibition. I_{50} were calculated from 10 points of a concentration curve, using regression analysis when the transformations were ln [y/(100-y)] = b lgx + const. for etamsylate and ln (100-y) = b lgx + const. for indomethacin.

for 30 min, the reaction mixture was acidified with 0.2 ml of 1.0 M HCl and extracted with 2 × 10 ml of ethyl acetate. The residue of the organic layer was spotted on silica gel G TLC-plates and developed with a mixture of ethyl acetate, acetic acid, iso-octane, and water in the ratio of 110:20:30:100 by volume as the solvent system, with the authentic standards $PGF_{2\alpha}$, PGE_2, 6-oxo-$PGF_{1\alpha}$, TXB_2, and arachidonic acid. The PGs and arachidonic acid were scanned for radioactivity (fig. 1). The R_f-values were: 6-oxo-$PGF_{1\alpha}$ 0.19; $PGF_{2\alpha}$ 0.28; PGE_2+TXB_2 0.41; and arachidonic acid 0.89. The PGs identified and the arachidonic acid were transferred to a scintillation vial and the radioactivity determined using a liquid scintillation counter. The activity of PG synthetase was calculated as pmole of PG formed per 30 min per mg of protein.

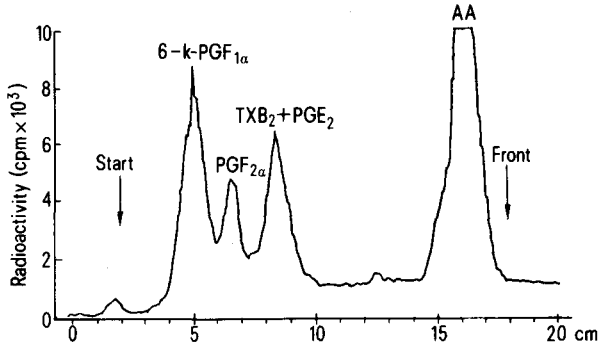


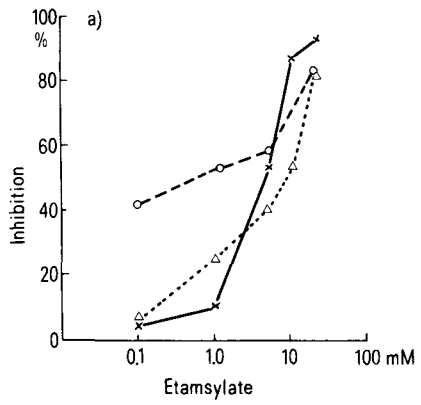
Figure 1. Thin-layer radiochromatogram of compounds isolated after incubation of [1-^{14}C] arachidonic acid with microsomes of pregnant human myometrium.

Results. Inhibition of PG synthesis in microsomes of pregnant human myometrium by etamsylate and indomethacin respectively is illustrated in figures 2a and 2b. Etamsylate inhibited synthesis of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, and TXB_2. The greater the concentration of etamsylate the greater was the inhibition of synthesis, although the curve for 6-oxo-$PGF_{1\alpha}$ production shows 2 plateaux of inhibition. The greater potency of indomethacin than of etamsylate in our assay system is shown in the table.

Discussion. The above results indicate that etamsylate exerts a potent inhibitory effect on the human pregnant myometrial PG synthesis in vitro. They support the findings of Bhattacherjee et al.[3] with respect to the inhibitory effect of etamsylate on PG biosynthesis in the rabbit.

Inhibition by etamsylate, expressed in I_{50}-values, is different for the different metabolites of arachidonic acid (table, fig. 2a). In addition, its mechanism is different from that whereby the non-steroid anti-inflammatory drugs such as indomethacin inhibit PG biosynthesis.

PG synthetase inhibitors such as indomethacin and other aspirin-like drugs prevent PG generation by direct inhibition of the enzyme cyclo-oxygenase that is responsible for PG biosynthesis[11]. This prevents formation of the first intermediate PG endoperoxides and, in consequence, of the PGs into which the endoperoxides would normally have been converted. According to our hypothesis etamsylate, in contrast to the aspirin-like drugs, has no anti-cyclo-oxygenase activity and does not affect the first step in PG biosynthesis (endoperoxide production), but acts by inhibiting the activity of endoperoxide reductase (for $PGF_{2\alpha}$), endoperoxide isomerase (for PGE_2), prostacyclin synthetase (for PGI_2), and thromboxane synthetase (for TXA_2). The effect of this different inhibition on the conversion of cyclic endoperoxide into further metabolites results in a different production of the PGs, as is shown by their different I_{50}-values (table).



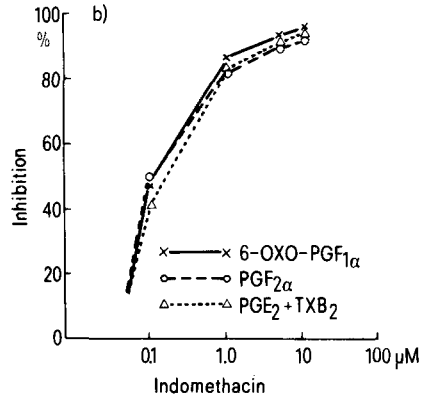


Figure 2. Inhibition of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$, and PGE_2+TXB_2 synthesis during incubation with etamsylate (a) and indomethacin (b). The sum of the PGE_2 and TXB_2 formed is shown in a single curve since these metabolites had the same mobility in our system. The conditions of incubation are described in the text. Each value represents duplicate experiments (variation 4–6%).

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