before superfusing the first RSS and RbA. After reoxygenation, the Krebs solution passed through a second coil (volume 20 ml) so that there was a 4 min delay before reaching the second RSS and RbA. Infusions of prostaglandin E_2 (20–80 ng/ml) or prostaglandin $F_{2\alpha}$ (40–160 ng/ml) into the first coil contracted the RSS in both banks of tissues, but had little effect on the rabbit aortas. When the enzyme preparation (0·1–0·4 mg protein/ml) was infused into the first coil, contractions of both RSSs showed the presence of a prostaglandin-like substance. Contractions of both RbAs also suggested the presence of RCS. Indomethacin (1 μg/ml) or meclofenamate (1 μg/ml) was then infused into the second coil. Now, when the enzyme preparation was infused into the first coil, the first RSS and RbA contracted, as did the second RSS. However, the contraction of the second RbA was reduced, showing that production of the rabbit-aorta contracting substance was inhibited and the activity detected by the first RbA had declined.

Thus, the enzyme preparation which generates prostaglandins from arachidonic acid also generates a rabbit-aorta contracting substance. The generation is inhibited by indomethacin or meclofenamate and the substance is unstable. All these characteristics fit with those of RCS which may, therefore, be an unstable intermediate in prostaglandin biosynthesis. One possibility is the cyclic endoperoxide postulated as the immediate common precursor of prostaglandins E and F (Samuelsson, Granström & Hamburg, 1967; Nugteren, Beerthius & Van Dorp, 1967).

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REFERENCES

ÄNGGÅRD, E. & SAMUELSSON, B. (1965). Biosynthesis of prostaglandins from arachidonic acid in guinea-pig lung. *J. biol. Chem.*, **240**, 3518–3521.

NUGTEREN, D. H., BEERTHIUS, R. K. & VAN DORP, D. A. (1967). Biosynthesis of prostaglandins. In: *Proc. II Nobel Symp.*, ed. Bergström, S. & Samuelsson, B. pp. 45–50. Stockholm.

PIPER, P. J. & VANE, J. R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature, Lond.*, **223**, 39–33.

PIPER, P. J. & VANE, J. R. (1971). The release of prostaglandins from lung and other tissues. *Ann. N.Y. Acad. Sci.*, **180**, 363–385.

SAMUELSSON, B., GRANSTRÖM, E. & HAMBURG, M. (1967). On the mechanism of biosynthesis of prostaglandins. In: *Proc. II Nobel Symp.*, ed. Bergström, S. & Samuelsson, B., pp. 31–44. Stockholm.

VANE, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biology*, **231**, 232–235.

VARGAFTIG, B. B. & DAO, N. (1971). Release of vaso-active substances from guinea-pig lungs by slow reacting substance C and arachidonic acid: its blockade by nonsteroidal anti-inflammatory agents. *Pharmacology*, in the Press.

Effect of sex hormones on the concentrations of plasma kininogen in the female rat

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Earlier studies in this laboratory have confirmed the observations of Wiegerhausen, Kläusch, Hennighausen & Sosat (1967) that the concentration of plasma kininogen rises with advancing gestation in the rat. This communication describes experiments to study the effect of oestrogens, progestagens and testosterone on non-pregnant female rats in an attempt to elucidate this phenomenon.

All drugs were administered to mature, virgin, female rats by the subcutaneous route. The concentrations of plasma kininogen were determined using the micromethod of Diniz & Carvalho (1963).

Stilboestrol (25 μg/kg and 50 μg/kg), oestradiol benzoate (5 μg/kg and 25 μg/kg) and ethinyl oestradiol (5 μg/kg and 10 μg/kg), administered daily for 5 days, produced significant increases in the concentrations of plasma kininogen; higher doses of stilboestrol (250 μg/kg) and ethinyl oestradiol (25 μg/kg) were without effect. Estrone hemisulphate (25 μg/kg and 125 μg/kg) administered daily for 5 days did not affect the concentrations of plasma kininogen. Administration of progesterone (500 μg/kg and 2·5 mg/kg) daily for 5 days, and 5 mg/kg twice daily for 5 days did not affect the concentrations of plasma kininogen. However, norgestrel (500 μg/kg and 2·5 mg/kg) daily for 5 days produced a significant rise in the concentration of kinin precursor. Administration of testosterone propionate (50 μg/kg, 250 μg/kg and 500 μg/kg) produced a significant fall in plasma kininogen content at all three dose levels.

Further experiments were performed using ovariectomized rats. The concentrations of plasma kininogen 5 days after ovariectomy were significantly lower than those found in intact, sham operated animals. There was no evidence that the concentrations of plasma kininogen were returning to normal 30 days after ovariectomy. Administration of stilboestrol (50 μg/kg) to ovariectomized rats, daily from the day of ovariectomy, did not raise the concentrations of plasma kininogen after treatment for 5 days but after treatment for 12 days the kininogen concentrations had increased to around the values found in intact female rats. Daily injection of progesterone (500 μg/kg) to ovariectomized rats from the day of ovariectomy did not raise the kininogen concentrations back to values found in intact female rates, even after treatment for 12 days.

These results show that oestrogens in optimal doses raised the concentrations of plasma kininogen in the female rat, and testosterone in all doses caused a fall in these concentrations. Progesterone produced no effect, but norgestrel significantly raised the concentration of plasma kininogen. It would appear from the experiments on ovariectomized animals that oestrogens are involved in maintaining normal concentrations of plasma kininogen in the female rat.

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REFERENCES

DINIZ, C. R. & CARVALHO, I. F. (1963). A micromethod for the determination of bradykininogen under several conditions. *Ann. N.Y. Acad. Sci.*, **104**, 77.

WIEGERHAUSEN, B., KLÄUSCH, G., HENNIGHAUSEN, G. & SOSAT, R. (1967). Der kininogenspiegel von ratten und kaninchen während der gestation. *Experientia, Basel*, **24**, 1128.

Oestradiol binding in hypothalamic cytosol

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It is believed that in oestrogen target cells oestradiol is transported to the nucleus in the form of high affinity complexes with cytosol protein. Oestradiol binding protein in hypothalamic cytosol has been reported (Eisenfeld, 1970) and this reaction may trigger feedback effects on gonadotrophin release.

These experiments compare (a) the distribution of high affinity oestradiol binding in cytosols from hypothalamus, amygdala, cerebral cortex and cerebellum, (b) the properties of hypothalamic and uterine binding protein, and (c) the number of available high affinity sites in the hypothalamus in relation to maturity, gender and the sexual cycle.