was also augmented, sometimes in height and always in duration. No prostaglandin release was detected during indomethacin infusion.

In three experiments when the change in spleen weight and perfusion pressure induced by splenic nerve stimulation had been increased by indomethacin, infusions of prostaglandin E_2 (1·5–5 ng/ml) into the spleen decreased the effects of nerve stimulation.

After cessation of indomethacin infusion, changes in weight, perfusion pressure and prostaglandin release induced by nerve stimulation gradually returned towards pretreatment levels.There was also a reduction in basal perfusion pressure which was associated with an increase in the continuous output of prostaglandins.

These experiments support Hedqvist's hypothesis that in the spleen, prostaglandin release is a feed-back mechanism which limits the effects of nerve stimulation. This is especially true for the duration of the effect. In addition our results show that there is a continuous basal release of prostaglandins which affect the perfusion pressure by actively causing vasodilatation.

We wish to thank the Wellcome Trust for a grant.

REFERENCES

FERREIRA, S. H., MONCADA, S. & VANE, J. R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature, New Biology*, **231**, 237–239.

HEDQVIST, P. (1970). Control of prostaglandin E_2 on sympathetic neurotransmission in the spleen. *Life Sci.*, part I, 269–278.

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

Rabbit-aorta contracting substance (RCS) may be a prostaglandin precursor

R. GRYGLEWSKI* and J. R. VANE

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

Rabbit-aorta contracting substance (RCS) is an unstable principle released from guinea-pig isolated lungs during anaphylaxis, and by infusions of arachidonic acid (Piper & Vane, 1969, 1971; Vargaftig & Dao, 1971). The release of RCS and prostaglandins is inhibited by aspirin and indomethacin (Piper & Vane, 1969, 1971; Vane, 1971). Vane (1971) equated prostaglandin release with biosynthesis and demonstrated that aspirin and indomethacin inhibit enzymic synthesis from arachidonic acid of prostaglandin E_2 and prostaglandin $F_{2\alpha}$. Because these results suggest that RCS may be related to prostaglandins, we have looked for RCS formation by a prostaglandin synthetase system.

The enzyme preparation was a washed particulate fraction (10,000–100,000 g) of homogenate of dog spleen. It was incubated for 20 min at 37°C with arachidonic acid (10 μg/ml) in a medium (Änggård & Samuelsson, 1965) containing glutathione (50 μg/ml) and hydroquinone (0·5 μg/ml); the generation of prostaglandins was 100–400 ng (assayed as prostaglandin E_2) per mg protein.

Samples of incubation mixture did not contract strips of rabbit aorta but RCS is very unstable. To test more immediately for RCS generation, two banks of assay tissues, each containing a strip of rat stomach (RSS) and rabbit aorta (RbA) were superfused at 5 ml/min with Krebs solution containing arachidonic acid (5 μg/ml). The Krebs solution passed through a coil (volume 10 ml) of silicone tubing at 37°C

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).

We thank the Wellcome Trust for a grant.

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

J. T. MCCORMICK* and JUDITH SENIOR
Department of Pharmacology, University of Bradford, Bradford 7

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).