The inhibitory effect of insulin on pinnal anaphylaxis in the mouse

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Insulin has been reported to increase the severity of systemic anaphylaxis in the rat, mouse and guinea-pig (Dhar, Sanyal & West, 1967a; Adamkiewicz, Sacra & Ventura, 1964), but to have no effect upon a local anaphylactic reaction in the rat paw (Dhar, Sanyal & West, 1967b). The results presented are from experiments undertaken to investigate the effect of insulin upon the development of a local anaphylactic reaction in the mouse pinna.

Male mice were sensitized to horse serum by subcutaneous injection of 0.1 ml of a $\frac{1}{20}$ dilution and 14 days later 0.1 ml of a $\frac{1}{50}$ dilution. Six to eight days after the second dose of horse serum the mice were taken to a warm room (30-32° C) and 1 h later were injected intravenously with Evans blue. Forty minutes later the mice were challenged, under ether anaesthesia, by piercing each ear through a drop of horse serum. A further 30 min later the mice were sacrificed and their ears removed and mounted on cards. The anaphylactic reaction was measured as the area of blueing around the site of challenge.

Insulin (Insulin B.P., Burroughs Wellcome & Co.), given intraperitoneally 40 min before challenge, in the dose range 0.1-20.0 i.u./kg, gave both a dose-related inhibition of the anaphylactic reaction and a dose-related hypoglycaemia. Glibenclamide (2-50 mg/kg p.o.) induced hypoglycaemia and an inhibition of the anaphylactic reaction.

Alloxan (100 mg/kg i.v.) induced severe hyperglycaemia and inhibited the anaphylactic reaction. Insulin, given to alloxan diabetic mice, produced animals with a normal serum glucose level but in which the anaphylactic reaction was markedly inhibited.

The inhibitory effect of insulin upon the anaphylactic reaction was reduced by surgical adrenalectomy and by the administration of the steroid synthesis inhibitor, metyrapone (2 x 200 mg/kg s.c.). The effect of insulin was unaffected by the α-adrenoreceptor blocking agent, phentolamine (1 or 5 mg/kg i.v.), but was reversed by the β-adrenoreceptor blocking agent, propranolol (25 mg/kg i.p.). With the exception of a slight reductive effect of propranolol, these treatments did not affect the hypoglycaemic response to insulin.

Histamine, 5-hydroxytryptamine and compound 48/80 induced a concentration-related blueing in the pinnae of non-sensitized mice when the ears were stabbed through solutions of these compounds. Pretreatment of the mice with insulin (10 i.u./kg i.p.) reduced the reaction induced by these compounds.

In pinnal anaphylaxis insulin has an inhibitory effect upon the development of the reaction. This effect is independent of its hypoglycaemic effect and appears to be mediated mainly through the adrenal glands.

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Muscular work and prostaglandin release

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Exercise is accompanied and followed by an increase in muscle blood flow. The mechanism of this functional hyperaemia is unclear, although chemical factors seem to be primarily involved (Mellander & Johansson, 1968).

We have measured the output of prostaglandin-like substances (PLS) into the venous blood from hind-legs in 38 vagotomized dogs, anaesthetized with a urethane-chloralose (0.37 g/kg and 75 mg/kg) mixture. PLS activity was measured by the blood-bathed organ technique (Vane, 1964). A rat stomach strip, chick rectum and rat colon were superfused in series at 10 ml/min with re-oxygenated blood from a femoral vein. These tissues

are contracted by low concentrations of prostaglandins of the E and F series, the rat colon being more sensitive to F's than to E's (Ferreira & Vane, 1967). Pretreatment of the assay tissues for 2 h (before bathing them in blood) with indomethacin (5 μg/ml) and phenoxybenzamine (2 μg/ml) increased their sensitivity to and specificity for prostaglandins (Gilmore, Vane & Wyllie, 1968). Muscular work was produced by electrical stimulation of the distal end of the cut sciatic nerve at 4 Hz (pulses of 5-7 v., 0.1 ms duration) for 5-10 minutes.

Stimulation of the sciatic nerve was often accompanied by the release into the femoral venous blood of an unidentified substance which relaxed the assay tissues. This relaxation waned and was superseded, usually during the fifth minute of exercise, by contractions of the assay tissues (45/51 trials). The contractions, indicative of PLS release, were sustained for up to 20 min after the exercise was over. Gallamine (3 mg/kg i.v.) prevented both muscle contraction and PLS output which could then be obtained by direct stimulation of the muscles. PLS release was also demonstrated in 6 experiments in which the vascularly isolated gracilis muscle was stimulated either via the gracilis nerve, or directly after gallamine.

The relative contractions of the assay tissues suggested that the PLS was mainly of the E series; the concentration released (assayed as prostaglandin E_2) ranged from 0.5-4.5 ng/ml (mean ± s.e. = 2.18 ± 0.24 ng/ml). When the large increase in blood flow which accompanied the muscle work is taken into account, it is evident that the output of PLS per min was substantial.

Indomethacin (2 mg/kg i.v.), an inhibitor of prostaglandin biosynthesis (Vane, 1971) was given in 17 dogs. Muscular exercise was no longer accompanied by PLS output, re-enforcing the conclusion that the contractor substance detected was a prostaglandin. The initial relaxation was still present. Thus PLS is released as a consequence of muscle contraction.

Prostaglandins of the E-series are potent vasodilators in the hind limb, so that this release may contribute to the functional hyperaemia. The fact that the release outlasts the exercise suggests that PLS contributes more to post-exercise hyperaemia than to that which occurs during exercise.

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Modulation of frequency-dependent noradrenaline release by calcium, angiotensin and morphine

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Adrenergic transmitter release appears to be regulated by a number of mechanisms at the site of release. These mechanisms may vary at different neuroeffector sites giving rise to different patterns of transmitter release in various tissues. Thus in the rabbit portal vein and vas deferens, when the external calcium ion concentration was 2.54 mM, the output/pulse of noradrenaline increased with the frequency of nerve stimulation whereas in the cat nictitating membrane and mouse vas deferens, the output/pulse was constant over the frequency range 0.2-15 Hz. These differences were not due to variations in transmitter uptake or metabolism (Hughes, 1972; Henderson & Hughes, unpublished observations). Further clarification of the mechanisms underlying the frequency-output relationship may be achieved by studying agents which modify the relationship.

All the experiments were carried out on isolated tissues bathed in Krebs solution at 36 ±1° C. Noradrenaline output was determined by measuring the overflow into the bathing fluid (Hughes, 1972).

When the external calcium concentration was increased from 2.54 to 5.08 mM, the output of noradrenaline from the rabbit vas deferens was preferentially increased at low frequencies of