The effects of vasopressin, adrenaline and noradrenaline on the mouse foetus.

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The effects of vasopressin (Pitressin) on the mouse foetus have been investigated following intra-amniotic injections (0·1–5 m-u.) into 15- or 16-day pregnant mice. Many of the foetuses surviving the effects of such an injection were abnormal when examined 1–4 days later. The abnormalities consisted of haemorrhagic lesions of the foetal extremities (limbs, tail, tongue and snout) and this was followed by necrosis and sometimes by congenital amputation of the extremity distal to the necrosis. The extent and location of the lesions depended on the dose of vasopressin administered: relatively low doses (for example, 0·1m-u.) affected the digits, while larger doses affected the whole limb, tail and snout in that order.

Adrenaline (5–10 μg) and noradrenaline (5–10 μg) produced effects on the foetus similar to those of vasopressin (0·5–1 m-u.), whereas isoprenaline (10 μg), 5-hydroxytryptamine (10 μg) and oxytocin (Pitocin, 5 m-u.) had no deleterious effects on the foetus when given by intra-amniotic injection.

The response of the foetal limb blood vessels to vasopressin, adrenaline and noradrenaline and the development of haemorrhages in the limbs was then observed directly under a dissecting microscope in the transilluminated foetuses of anaesthetized mothers. Subcutaneous injections of vasopressin (10–100 μ-u.), adrenaline (0·1–1·0 μg) and noradrenaline (0·1–1·0 μg) into the foetus produced intense arterial constriction in the foetal limbs with accompanying stagnation of blood in the limb veins. Following an intra-amniotic injection of 1 m-u. vasopressin, this arterial constriction persisted for at least 3 hr. After 4 hr, the distal branches of the main limb artery were still intensely constricted and haemorrhages appeared in the limb at the sites of bifurcation of the main limb artery proximal to the constriction. Similar results were obtained with intra-amniotic adrenaline or noradrenaline (5 μg).

These results indicate that prolonged and intensive arterial vasospasm is probably responsible for the production of haemorrhagic lesions in the foetal extremities. It is possible that as a result of prolonged vasoconstriction, ischaemic injury of the limb blood vessels and supporting tissues occurs. Thus, when the main limb artery proximal to the sites of bifurcation begins to relax, while marked constriction of the distal branches of this vessel is still present, a rise in pressure occurs in the damaged vessel, causing it to rupture at its weakest point (the sites of bifurcation).

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An attempt to determine whether acetylcholine can release acetylcholine from a sympathetic ganglion.

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It has been suggested by Koelle (1961) that, in sympathetic ganglia, the acetylcholine initially released by the preganglionic nerve impulse acts principally on the presynaptic terminals, to liberate a further charge of acetylcholine which then effects synaptic

transmission. If this were so, it might be expected that injected acetylcholine would also release acetylcholine from presynaptic terminals.

This prediction has been tested by labelling ganglionic acetylcholine with tritium and then observing whether stimulation of the ganglion by injection of unlabelled acetylcholine was accompanied by the release of ^{3}H-acetylcholine.

Cats were anaesthetized with chloralose. The superior cervical ganglion was perfused with eserinized Locke solution by Perry's (1953) method, with the modification of intermittent perfusion with the cat's own blood, which reduces ganglionic oedema (Jones & Quilliam, 1967). Ganglionic responses were monitored by recording the contraction of the nictitating membrane.

First, ganglionic acetylcholine was labelled by perfusing with eserinized Locke solution containing ^{3}H-choline (1 μg/ml) while stimulating the preganglionic nerve for 12 min at 10 or 20 Hz. The perfusion fluid was then changed to one containing unlabelled choline and thereafter the efflux of tritium monitored.

With each subsequent burst of preganglionic nerve stimulation (0·5 ms pulse width, 5–10 V, 10 Hz for 1 or 3 min duration) there was a substantial increase in tritium efflux. Electrophoretic separation of effluent acetylcholine and choline (Potter & Murphy, 1967) showed this increased efflux to stem entirely from release of ^{3}H-acetylcholine.

K^{+} ions can release acetylcholine from sympathetic ganglia (Brown & Feldberg, 1936). Injection of K^{+} ions into the perfusion stream increased effluent radioactivity, and also reduced the effect of subsequent nerve stimulation.

By contrast, effects of acetylcholine and other nicotinic stimulants (carbachol, tetramethylammonium, nicotine) on tritium efflux were usually negligible even when ganglionic responses as great as those elicited by nerve stimulation were obtained.

Increases in effluent radioactivity were occasionally observed after injections of carbachol or acetylcholine, but this effect differed in several respects from that of nerve stimulation: (*a*) It was not related to the degree of ganglion stimulation but seemed to reflect an increased perfusion flow rate. (Methacholine, which also increased flow rate but did not stimulate the ganglion, likewise augmented tritium efflux.) (*b*) The effluxes of *both* ^{3}H-acetylcholine and ^{3}H-choline were increased. (*c*) The efflux of ^{14}C-sucrose or ^{35}S-sulphate was also increased. This suggests that such effects were due to washout of extracellular radioactive material following ganglionic vasodilatation, rather than to release of neuronal acetylcholine.

These experiments indicate that ganglionic presynaptic terminals are not readily stimulated by acetylcholine. In this respect, they do not support the hypothesis of a dual pre- and post-synaptic neurohumoral function for acetylcholine in the sympathetic ganglion.

This study was aided by grants from the Governors of St. Bartholomew's Hospital and from the American Medical Association Education and Research Foundation.

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The action of β-receptor antagonists on intracellular cardiac potentials.

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Dohadwalla, Freedberg & Vaughan Williams (1969) have reported that in isolated rabbit atria, 60 min exposure to (−)-, (+)- and (±)-propranolol and practolol (I.C.I. 51072) greatly reduced the rate of rise (MRD) of the intracellularly recorded action potential at concentrations which had no significant effect on contractility, repolarization time and other cardiac parameters. They concluded that the MRD "was by far the most sensitive test of the activity of the drugs on cardiac function".

In the present experiments, (±)-propranolol ($1{\cdot}0\times10^{-5}$M) after 10 and 20 min exposure decreased the MRD and amplitude of the action potential and depressed contraction in rabbit atria, which agrees with the above results. However, in some experiments (±)-propranolol ($1{\cdot}0\times10^{-6}$M) caused a significant increase in MRD and action potential amplitudes in spite of a significant depression of the contractile response.

In other experiments, however, notably with 10^{-6}M propranolol on spontaneously beating guinea-pig atria, and with 10^{-5}M practolol on driven guinea-pig atria, MRD was reduced to a greater extent than contractions. Similar results were obtained with (±)-propranolol ($1{\cdot}0\times10^{-6}$M) in guinea-pig atria driven at 60/min (left atria) and at 180/min (combined right and left atria). At a higher concentration ($1{\cdot}0\times10^{-5}$M) of (±)-propranolol there were no differences in the electrophysiological parameters from control values in these preparations.

Practolol and oxprenolol, two other β-receptor blocking agents, had almost no effect (even at concentrations much greater than that necessary to cause substantial ß-receptor blockade) on MRD, action potential size, or configuration of the action potential when tested on guinea-pig left atria.

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The effects of intravenous acetylcholine on the cardiovascular system of the anaesthetized dog.

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The fall in systemic blood pressure following the intravenous administration of acetylcholine was found not to be associated with a direct effect of acetylcholine on resistance in the femoral vasculature.