Effects of cytochalasin B and colchicine on secretion of posterior pituitary and adrenal medullary hormones

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Lacy, Howell, Young & Fink (1968) found colchicine inhibits insulin secretion and suggested that microtubules or like entities regulate emiocytosis (exocytosis). Poisner & Bernstein (1971) drew similar conclusions from colchicine's inhibitory effect on acetylcholine (ACh) or nicotine-induced adrenal medullary secretion. Since neurohypophyseal secretion apparently involves exocytosis (Douglas, Nagasawa & Schulz, 1971 ; Nagasawa, Douglas & Schulz, 1971 ; Uttenthal, Livett & Hope, 1971) we have tested colchicine and cytochalasin, a drug associated with microfilaments (Wessels *et al.*, 1971) on the neurohypophysis, and compared responses with those of the adrenal.

Rat neurohypophyses, ten per experiment, were halved and two paired sets assembled. After 90 min preincubation, one set was exposed to cytochalasin (40 μg/ml) for 30 min and the other to the cytochalasin solvent, dimethylsulphoxide (0·4%). During the last 10 min exposure to the drugs the glands were stimulated with 60 mM K, or 2 mM Ca introduced into high K, Ca-free medium. Responses in cytochalasin were only 39 ± 2 and $37 \pm 5\%$ (mean $\pm$ S.E., n=4) of the controls. Similar inhibition by cytochalasin (to $44 \pm 2\%$) was observed with electrical stimulation. Colchicine also reduced responses to K but the effect was relatively feeble, to $72 \pm 5\%$ of controls.

Cats adrenal glands perfused retrogradely were stimulated for 1 min in each 20 min period. Cytochalasin (50 μg/ml) during, and 16 min before, stimulation reduced catecholamine secretion to ACh (10^{-5} g/ml) and K (60 mM) to $18 \pm 3\%$ and $56 \pm 4\%$ of the mean of preceding and following controls. The larger inhibition of ACh, suggesting partial involvement of receptors, prompted reinvestigation of colchicine which Poisner & Bernstein (1971) tested only against nicotine or ACh. The above protocol was followed, but with rabbit adrenals perfused arterially. Colchicine (5×10^{-4} M) reduced ACh-evoked responses to $46 \pm 9\%$ of the controls, in harmony with results on ox glands (Poisner & Bernstein, 1971) ; but responses to K were little affected ($95 \pm 2{\cdot}5\% \times$ controls ; n=4).

Since colchicine in high concentration reduced neurohypophyseal and medullary secretions to K little or not at all these responses are unlikely to depend critically on colchicine-sensitive components such as microtubules. Arguments to the contrary, based on inhibitory effects against ACh or nicotine-evoked medullary secretion (Poisner & Bernstein, 1971), must be discounted since these effects, in sparing responses to K, are hexamethonium-like and indicative of actions on receptors, rather than secretion proper. On the other hand, some cytochalasin-sensitive component is important for these secretions. During our study Schofield (1971) reported a first K-stimulated release of growth hormone was unaffected by cytochalasin (10 μg/ml) but a second was depressed. He suggested cytochalasin blocks centrifugal movement of granules to replenish release sites. Our finding of inhibition of *initial* responses indicates an additional action probably on exocytosis proper, and revealed perhaps by our use of higher doses. Possibly this involves microfilaments or other contractile elements. Alternatively, inhibition of responses to Ca, considered

along with the common calcium requirement of exocytoxic processes, could indicate depression of calcium influx. Such an action might also explain other effects of cytochalasin (Wessels *et al.*, 1971).

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Properties of a new prostaglandin

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It has been shown that PGA_1 and PGA_2 slowly lose their depressor activity during incubation with blood plasma of the cat. This has been attributed to an enzymic conversion of the PGA to its biologically-inactive isomer, PGB (Jones, 1970; Horton *et al.*, 1971). Further studies have established that the enzyme system, which has provisionally been given the name prostaglandin isomerase, causes a single shift of the 10,11 double bond of PGA_1 to produce the 9-oxo-11,13-diene isomer (Fig. 1). This new prostaglandin has been designated PGC_1. It is unstable, isomerizing to PGB_1 under mild alkaline conditions (>pH 7).

The isolation of small quantities of PGC_1 and PGC_2, free from the corresponding PGA and PGB, has been achieved. Initial observations indicated that these compounds were highly active depressor agents in the cat and dog. A more detailed investigation of the depressor effects in comparison with several other prostaglandins were therefore carried out. When injected rapidly into the thoracic aorta of the pentobarbitone-anaesthetized cat, prostaglandins E_1 (20 ng/kg) and B_1 (2 μg/kg) elicit smooth falls in diastolic B.P. which on average reach a maximum 15 sec after injection and have decreased to two-thirds maximum after 35 sec. PGC_1 (50 ng/kg) produces a more prolonged fall in B.P., reaching a maximum after 45 sec and declining to two-thirds maximum in 125 sec. In contrast, the response to PGA_1 (100 ng/kg) is biphasic; the initial fall in pressure reaches a maximum 15 sec after injection and is followed by a prolonged and more pronounced fall with a maximum at 85 sec.

By comparing the falls in B.P. 15 sec after injection, estimates of the potencies of the prostaglandins have been made. Relative to PGE_1 (=100), PGA_1, PGB_1 and PGC_1 have potencies of 16, 0·9 and 44 respectively. Similarly, PGA_2, PGB_2 and PGC_2 possess 16, 1·2 and 47% of the activity of PGE_2.