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COMMUNICATIONS

Evidence for inhibition of glucose transport in peripheral adrenergic neurons by cytochalasin B

There is currently considerable interest in the possible role of contractile processes in the release of transmitters, hormones and other secretory products from different tissues. The effects on such processes of cytochalasin B have been extensively examined (for references see Sorimachi, Oesch & Thoenen, 1973; Nakazato & Douglas, 1973) since it has been suggested that this compound acts by disrupting the function of microfilaments (Wessels, Spooner & others, 1971).

Cytochalasin B apparently impairs exocytosis in peripheral adrenergic neurons since it markedly reduced the release of [^{3}H]noradrenaline from guinea-pig atria by 60 mM K^+ and by nicotine (Sorimachi & others, 1973) and reduced the release of both noradrenaline and dopamine β-hydroxylase from guinea-pig vas deferens on sympathetic nerve stimulation (Thoa, Wooten & others, 1972). Sorimachi & others (1973) have cautioned that a causal relation between the effect on microfilaments and the impairment of exocytosis has not been demonstrated.

In perfused superior cervical sympathetic ganglia of the cat, cytochalasin B blocked transmission of nerve impulses, at least in part, by depressing energy production since its effects could be partially overcome by pyruvate (Nakazato & Douglas, 1973). These and other results led Nakazato and Douglas to reject the proposal that "functional changes induced by cytochalasin in secretory cells constitute proof of involvement of microfilaments." It is possible that cytochalasin B could impair energy production by reducing glucose transport. Certainly cytochalasin B inhibited the uptake of D-glucose and 2-deoxy-D-glucose by HeLa cells (Mizel & Wilson, 1972), leucocytes (Zigmond & Hirsch, 1972), fibroblasts and liver cells (Kletzien, Perdue & Springer, 1972) and uterine tissue (Gorski & Racker, 1973).

In the present study, the effects of cytochalasin B on [^{3}H]metaraminol accumulation by rabbit atria have been examined under both aerobic and anaerobic conditions. Under anaerobic conditions, normal accumulation of amine is critically dependent upon the provision and transport of D-glucose (Paton, 1972).

Pieces of rabbit atria were prepared as described previously (Paton, 1972) and preincubated at 37° for 30 min in a physiological salt solution. [^{3}H]metaraminol (1×10^{-8}M) was then added to the media and the incubation continued for a further 30 min. At the end of this period, the total [^{3}H] content of tissues was determined by liquid scintillation spectrometry as described previously (Paton, 1972). The net accumulation of [^{3}H]metaraminol was expressed as pmol g^{-1} wet weight. The medium used had the following composition (mM): NaCl, 140; KCl, 5; $CaCl_2$, 1·5; $MgSO_4$, 1·2; tris-HCl (pH 7·4), 10; Na_2 EDTA, 0·03; Na ascorbate, 0·1. The medium was equilibrated with either 100% O_2 or 100% N_2, and contained either 2·5 mM D-glucose or 2·5 mM sucrose as indicated.

Chromatographically pure (±)-metaraminol-7-[^{3}H] with specific activity of 6·72 Ci $mmol^{-1}$ was obtained from the New England Nuclear Corporation. Cytochalasin B (Imperial Chemical Industries, England) was dissolved in dimethylsulphoxide (DMSO), the stock solution containing 1 mg ml^{-1}. Consequently, when the effects of 10 μg ml^{-1} cytochalasin B were studied, 1% DMSO was present in the medium. The effects of 1% DMSO itself were therefore also examined.

It can be seen (Table 1) that 1% DMSO did not alter the net accumulation of [^{3}H]metaraminol under aerobic or anaerobic conditions. As reported previously (Paton, 1972), accumulation of [^{3}H]metaraminol was not reduced under anaerobic

Table 1. *Effect of cytochalasin B on the accumulation of [^3H] metaraminol by rabbit atria.* Tissues were incubated under the conditions indicated for 60 min, [^{3}H]metaraminol (1×10^{-8}M) being added for the final 30 min. Mean ± s.e. of 5–12 observations. $^*P < 0{\cdot}05$, compared to controls.

Incubation conditions				[^{3}H]-metaraminol accumulation (pmol g^{-1} wet weight)
O_2	D-Glucose (2·5mM)	DMSO (1%)	Cytochalasin B (10μg ml^{-1})	
+	+	—	—	70·8±5·2
+	+	+	—	72·8±2·9
+	+	+	+	68·2±2·9
—	+	—	—	73·8±7·4
—	+	+	—	70·3±5·4
—	+	+	+	21·4±1·6*
—	—	—	—	26·4±1·0*

conditions, provided that D-glucose was present as substrate. Cytochalasin B did not alter accumulation under aerobic conditions but very markedly reduced accumulation under anaerobic conditions to the levels found in anaerobic tissues deprived of external D-glucose. Sorimachi & others (1973) also demonstrated that, under aerobic conditions, the uptake of tyramine in guinea-pig atria was not impaired by cytochalasin B.

These studies provide strong evidence that cytochalasin B inhibits glucose transport in peripheral adrenergic neurons since, under anaerobic conditions (Paton, 1972), accumulation of amine requires the provision of D-glucose or D-mannose as substrate. It is less likely that cytochalasin B inhibited glycolysis directly since iodoacetic acid partially reduced amine accumulation even under aerobic conditions (Paton, 1972). These findings thus support the contention of Nakazato & Douglas & others (e.g., Anderson, Edström & Mattson, 1972) that the results of studies utilizing cytochalasin B must be interpreted with caution.

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REFERENCES

ANDERSON, K. E., EDSTRÖM, A. & MATTSSON, H. (1972). *Brain Res.*, **48**, 343–353.
GORSKI, J. & RACKER, B. (1973). *Endocrinology*, **93**, 1212–1215.
KLETZIEN, R. F., PERDUE, J. F. & SPRINGER, A. (1972). *J. biol. Chem.*, **247**, 2964–2966.
MIZEL, S. B. & WILSON, L. (1972). *Ibid.*, **247**, 4102–4105.
NAKAZATO, Y. & DOUGLAS, W. W. (1973). *Proc. Nat. Acad. Sci.* (*Wash.*), **70**, 1730–1733.
PATON, D. M. (1972). *Pharmacology*, **7**, 78–88.
SORIMACHI, M., OESCH, F. & THOENEN, H. (1973). *Arch. Pharmac.*, **276**, 1–12.
THOA, N. B., WOOTEN, G. F., AXELROD, J. & KOPIN, I. J. (1972). *Proc. Nat. Acad. Sci.* (*Wash.*), **69**, 520–522.
WESSELS, N. K., SPOONER, B. S., ASH, J. F., BRADLEY, M. P., LUDUENA, M. A., TAYLOR, E. L., WRENN, J. R. & YAMADA, K. M. (1971). *Science*, **171**, 135–143.
ZIGMOND, S. H. & HIRSCH, J. G. (1972). *Ibid.*, **176**, 1432–1434.