CYTOCHALASIN B, PITUITARY METABOLISM, AND THE RELEASE OF OX GROWTH HORMONE *IN VITRO*

M.A. McPHERSON and J.G. SCHOFIELD
Department of Biochemistry, University of Bristol, Bristol, BS8 1TD, England

Received 9 May 1972

1. Introduction

Cytochalasin B, a metabolite from *Helminthosporium dematioideum* which is believed to block the activity of intracellular contractile microfilaments [1], has recently been used to investigate the mechanism of emiocytosis. The inhibition of non-protein bound iodine [2], growth hormone [3], histamine [4] and norepinephrine [5] release, and the potentiation of insulin release [6], by cytochalasin B, have both been used to substantiate the hypothesis that contractile microfilaments are involved in secretion. This interpretation depends on the assumption that cytochalasin B is specific in its action. However, cytochalasin B inhibits glucose oxidation in platelets [7], and, at concentrations below those used in other systems to inhibit secretion, it inhibits glucosamine incorporation into mucopolysaccharides and glycoproteins [8]. In this report we show that the effect of cytochalasin B on growth hormone release depends on the exogenous substrate added; cytochalasin B inhibits prostaglandin E_2 induced growth hormone release in the presence of glucose alone, but potentiates release if β-hydroxybutyrate and glutamate are also added. Since cytochalasin B inhibits oxidation of glucose but not β-hydroxybutyrate without changing the tissue ATP concentration, and since glucose oxidation is more sensitive to cytochalasin B than is growth hormone release, it is possible that inhibition of release results from changes in pituitary metabolism rather than changes in microfilament activity.

2. Methods

Pituitary glands were obtained from heifers and incubated in Krebs–Henseleit bicarbonate buffered salt solution as described previously [9]. All incubation media contained glucose (5.5 mM), and in some experiments sodium β hydroxybutyrate (5.5 mM) and sodium glutamate (5.5 mM) were added. The slices were lightly blotted, weighed on a torsion balance, and preincubated for two successive 60 min periods in 1.5 ml medium to which a stock solution of cytochalasin B in dimethylsulphoxide (5 mg/ml) or an equal volume of dimethylsulphoxide had been added. They were then incubated for 60 min in 1.0 ml of the same medium with or without prostaglandin E_2 (1 μM).

At the end of this incubation the media were removed for measurement of growth hormone concentrations by radioimmunoassay [9], and the slices were quenched and homogenised in 0.5 ml H_2SO_4 (2.5 N) at 0°. The homogenate was centrifuged (3000 *g*, 10 min, 4°), and 10 μl supernatant removed and diluted into 40 μl saturated Na_3PO_4, (approx. 0.8 M) for measurement of ATP concentration by a modification of the method described by Stanley and Williams [10] using a firefly lantern extract containing luciferase (Sigma FLE-50). The remainder of the extract was deproteinised by addition of 0.5 ml barium acetate (1 M) followed by 0.8 ml saturated Na_3PO_4, and centrifuged (3000 *g*, 30 min, 4°). The resultant supernatant was stored at −15°, and the

concentration of cyclic 3′, 5′ AMP measured by the binding protein assay as described elsewhere [11] but using 0.2 M potassium phosphate adjusted to pH 6.5 instead of pH 5.5.

The effects of cytochalasin B on oxidation of [U-^{14}C] glucose or [3-^{14}C] DL β hydroxybutyrate were investigated in parallel experiments. The slices were preincubated in the presence or absence of cytochalasin B as described above, and then incubated for a further 60 min in 1 ml medium containing either [U-^{14}C] glucose (0.1 μCi, 5.5 mM) or [3-^{14}C] DL β hydroxybutyrate (0.1 μCi, 5.5 mM), glucose (5.5 mM) and glutamate (5.5 mM). Oxidation was stopped by addition of 0.5 ml H_2SO_4 (2 M) and $^{14}CO_2$ absorbed in hyamine (0.4 ml hyamine base) by shaking at room temperature for 120 min. The hyamine was diluted in 10 ml toluene scintillator for determination of radioactivity in a liquid scintillation counter.

3. Results and discussion

In the presence of glucose alone, cytochalasin B (10 μg/ml) decreased the prostaglandin E_2 induced rise in growth hormone release from 1.57 μg/mg/hr (table 1). The metabolism of [U-^{14}C] glucose to $^{14}CO_2$ was not altered by prostaglandin E_2. Hertelendy et al. [12] have reported that theophylline and dibutyryl cyclic AMP caused a 50% rise in pituitary production of $^{14}CO_2$ from [1-^{14}C] glucose, but in these experiments this effect would be masked by the presence of label in other glucose carbons. Cytochalasin B (10 μg/ml) caused a 90% inhibition of [U-^{14}C]-glucose oxidation to $^{14}CO_2$ (table 1). Inhibition of this magnitude might be expected to lower tissue ATP concentrations and hence decrease growth hormone release [13, 14], but ATP concentrations were not changed after 3 hr incubation in cytochalasin B. Cytochalasin B did not significantly alter the control or stimulated concentrations of cyclic AMP.

If inhibition of glucose oxidation results in inhibition of growth hormone release, then both parameters might be expected to be equally sensitive to cytochalasin B. It was found, however, that 2 μg/ml cytochalasin B caused 80% inhibition of [U-^{14}C] glucose oxidation to $^{14}CO_2$, without changing prostaglandin E_2 induced growth hormone release (table 2). It is therefore possible that cytochalasin B inhibits two separate processes with different affinities, although an alternative explanation is that normal growth hormone release can be maintained by glucose oxidation at not less than 20% of the normal rate.

If cytochalasin B inhibits release by interacting with microfilaments at higher concentrations than those necessary to inhibit glucose oxidation, then it should also inhibit release in the presence of substrates whose oxidation it does not alter. Table 3 shows that [3-^{14}C] β hydroxybutyrate oxidation to $^{14}CO_2$ is not affected by cytochalasin B. However, cytochalasin B potentiated the effect of prostaglandin E_2 on growth hormone release by pituitaries incubated in β hydroxybutyrate, glutamate and glucose. Williams and Wolff [2] reported that cytochalasin B (10 μg/ml) increased basal release of protein bound iodine from thyroid tissue, and suggested that this resulted from cell damage. Since neither the basal release of growth hormone nor the tissue ATP concentration was altered by cytochalasin B, potentiation of

Table 1
Effect of cytochalasin B (10 μg/ml) on pituitary slices incubated in the presence of glucose.

Incubation conditions	Rate of growth hormone release (μg/mg wet/hr)	[U-^{14}C] glucose metabolised to $^{14}CO_2$ (μmoles/g wet/hr)	ATP concentration (μmoles/g wet)	Cyclic AMP concentration (nmoles/g wet)
Control	0.60 ± 0.05 (49)	0.81 ± 0.09 (8)	0.84 ± 0.10 (26)	0.27 ± 0.04 (13)
Prostaglandin E_2	2.17 ± 0.23	0.76 ± 0.08	0.82 ± 0.05	1.15 ± 0.13
Cytochalasin B	0.54 ± 0.05	0.09 ± 0.01 [b]	0.79 ± 0.05	0.44 ± 0.08
Cytochalasin B + Prostaglandin E_2	1.06 ± 0.07 [a]	0.06 ± 0.02 [a]	0.70 ± 0.05	0.84 ± 0.10

a: $P < 0.001$ versus prostaglandin E_2; b: $P < 0.001$ versus control.

Table 2
Effect of cytochalasin B (2 μg/ml) on pituitary slices incubated in the presence of glucose.

Incubation conditions	Rate of growth hormone release (μg/mg wet/hr)	[U-^{14}C] glucose metabolised to $^{14}CO_2$ (μmoles/g wet/hr)
Control	0.28 ± 0.04 (16)	0.69 ± 0.05 (16)
Prostaglandin E_1	0.66 ± 0.09	0.63 ± 0.07
Cytochalasin B	0.31 ± 0.04	0.14 ± 0.02 [b]
Cytochalasin B + Prostaglandin E_2	0.70 ± 0.08	0.14 ± 0.02 [a]

a: $P < 0.001$ versus prostaglandin E_2; b: $P < 0.001$ versus control.

Table 3
Effect of cytochalasin B (10 μg/ml) on pituitary slices incubated in the presence of glucose, β hydroxybutryate and glutamate.

Incubation conditions	Rate of growth hormone release (μg/mg wet/hr)	[3-^{14}C] β hydroxybutyrate metabolized to $^{14}CO_2$ (μmoles/g wet/hr)	ATP concentration (μmoles/g wet)	Cyclic AMP concentration (nmoles/g wet)
Control	0.50 ± 0.08 (24)	1.12 ± 0.15 (8)	0.80 ± 0.07 (20)	0.22 ± 0.04 (10)
Prostaglandin E_2	1.18 ± 0.20	1.45 ± 0.14	0.75 ± 0.05	0.85 ± 0.13
Cytochalasin B	0.57 ± 0.08	1.40 ± 0.24	0.81 ± 0.09	0.32 ± 0.03
Cytochalasin B + Prostaglandin E_2	1.82 ± 0.18 [a]	1.33 ± 0.26	0.79 ± 0.04	2.11 ± 0.28 [b]

a: $P < 0.02$ versus prostaglandin E_2; b: $P < 0.001$ versus prostaglandin E_2.

release was probably not caused by non-specific cell damage. It might however be related to the potentiation of the prostaglandin induced rise in tissue cyclic AMP concentration (table 3).

We have previously reported [3] that in the presence of glucose cytochalasin B inhibited the effect of high K^+ and prostaglandin E_2, but not Ba^{2+}, on growth hormone release. We report here that cytochalasin B can either inhibit or potentiate prostaglandin induced growth hormone release depending on the substrate used, and that glucose metabolism is more sensitive to inhibition by cytochalasin B than is hormone release. It has been argued that cytochalasin B directly inhibits histamine release by inhibiting microfilament contraction [4], and that it directly potentiates insulin release by disrupting a microfilamentous barrier to secretion [5]. Our observation that substrates whose oxidation is not inhibited by cytochalasin B are able to alter its effect on release from inhibition to potentiation suggests that the effect is at least in part on metabolism. It may be relevant to note that Hertelendy et al. [12] reported that theophylline and dibutyryl cyclic AMP increased oxidation of glucose in the rat pituitary by increasing flow through the pentose phosphate pathway; it is not yet known whether cytochalasin B inhibits formation of glucose 6 phosphate in bovine anterior pituitary.

Acknowledgements

This work was supported by grants from the British Diabetic Association and the British Insulin Manufacturers to Professor P.J. Randle, whom we thank for his interest and advice during the work. M.A.McP. holds a Medical Research Council Scholarship for training in research methods. E_2 was a gift from Dr. J.E. Pike of Upjohn Ltd., Kalamazoo, Mich., U.S.A.

References

[1] N.K. Wessels, B.S. Spooner, J.F. Ash, M.O. Bradley, M.A. Luduena, E.L. Taylor, J.T. Wren and K.M. Yamada, Science 171 (1971) 135.
[2] J.A. Williams and J. Wolff, Biochem. Biophys. Res. Commun. 44 (1971) 422.
[3] J.G. Schofield, Nature New Biology 234 (1971) 215.
[4] T.S.C. Orr, D.E. Hall and A.C. Allison, Nature 236 (1972) 350.
[5] N.B. Thoa, G.F. Wooten, J. Alexrod and I.J. Kopin, Proc. Natl. Acad. Sci. U.S. 69 (1972) 520.
[6] L. Orci, K.H. Gabbay and W.J. Malaisse, Science 175 (1972) 1128.
[7] R.J. Haslam, Biochem. J. 127 (1972) 34p.
[8] J.W. Sanger and H. Holtzer, Proc. Natl. Acad. Sci. U.S. 69,(1972) 253.
[9] J.G. Schofield, Biochem. J. 103 (1967) 331.
[10] P.E. Stanley and S.G. Williams, Anal. Biochemistry 29 (1969) 381.
[11] R.H. Cooper, M.A. McPherson and J.G. Schofield, Biochem. J. 127 (1972) 143.
[12] F. Hertelendy, G. Peake and H. Todd, Biochem. Biophys. Res. Commun. 44 (1971) 253.
[13] J.G. Schofield and M. Steed, FEBS Letters 13 (1971) 149.
[14] F. Hertelendy, Acta Endocrinologica 68 (1971) 355.