INHIBITION OF ANTERIOR PITUITARY CYCLIC AMP PHOSPHODIESTERASE BY COLCHICINE AND VINBLASTINE

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1. Introduction

Secretory processes involve migration of stored material, often in granular form, to the cell surface for release and microtubules have been implicated in the mechanism of hormone secretion from the pancretic B-cell [1], the thyroid [2] and the adrenal medulla [3]. A universal role for microtubules in hormone secretion has, however, not been established. Thus, the microtubule inhibitor, colchicine, does not inhibit the secretion of thyroid stimulating [4], adrenocorticotrophic [5] or growth hormones [6–8] from the rat anterior pituitary. During studies to elucidate further the possible role of microtubules in hormone secretion, an unexpected effect of the microtubule inhibitors, notably inhibition of anterior pituitary cyclic AMP phosphodiesterase, was observed and is reported here.

2. Experimental

2.1. *Materials*

Colchicine, theophylline, 5′-nucleotidase, QAE-Sephadex, cyclic AMP and its dibutyryl derivative were from Sigma and vinblastine sulphate from Eli Lilly. Prostaglandin E_2 was the kind gift of Dr John Pike of Upjohn. New England Nuclear supplied [α-^{32}P]ATP (2 Ci/μmol). Cyclic AMP assay kits and cyclic [^{3}H]AMP were obtained from Amersham. Other reagents were of the highest purity commercially available.

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2.2. *Assay procedures*

Anterior pituitary fragments were obtained from 200 g male Sprague-Dawley rats and rates of growth hormone release were determined as in [6]. Assay of anterior pituitary cyclic AMP concentration was done as in [9]. Pituitary adenylate cyclase and cyclic AMP phosphodiesterase were determined as in [10,11].

3. Results

During the course of 3 successive 1 h incubations in vitro, no effect of colchicine or vinblastine upon growth hormone release was seen until 10^{-3} M colchicine and 10^{-4} M vinblastine were attained. At these concentrations, paradoxical stimulatory effects of the agents were observed during the third hour of incubation, a significant effect of vinblastine being seen only in the presence of the known stimulator of growth hormone secretion, dibutyryl cyclic AMP (table 1). Calcium deprivation and the addition of 2,4-dinitrophenol (2.5×10^{-4} M) abolished the stimulatory effect of colchicine seen during the third hour of incubation (table 2) and this evidence of calcium- and energy-dependency was interpreted as evidence of the true secretory nature of the colchicine effect.

Since pituitary concentrations of cyclic AMP are known to be the main determinant of rates of rat growth hormone secretion [6,12], the effects of colchicine (10^{-3} M) and vinblastine (10^{-4} M) on the concentration of the cyclic nucleotide were examined. Both alkaloids led to statistically significant increases in pituitary cyclic AMP concentration as did theophylline (5×10^{-4} M) and prostaglandin E_2 (10^{-6} M) included as positive controls (table 3). To identify the mechanism of this effect of the alkaloids, their influ-

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Table 1
Effects of colchicine and vinblastine upon rates of growth hormone release in vitro (μg NIAMDD-rat GH-RP-1 . h^{-1} . mg wet wt^{-1})

Inhibitor	Dibutyryl cyclic AMP in medium	
	0 mM	1 mM
None [control]	2.34 ± 0.13 (15)	4.03 ± 0.26 (21)
Colchicine (10^{-3} M)	3.64 ± 0.17 (18)[a]	4.98 ± 0.33 (24)[a]
None [control]	2.31 ± 0.30 (15)	4.09 ± 0.19 (26)
Vinblastine (10^{-4} M)	2.96 ± 0.33 (15)	5.99 ± 0.43 (26)[a]

[a] $p < 0.05$ *vs* corresponding control

Rates of growth hormone secretion were determined as in section 2 during the third hour of incubation under the experimental conditions indicated

Results are expressed as means ± SEM of no. obs. in parentheses

ence on rates of cyclic AMP formation and breakdown were investigated. Employing a 10^{-4} M ATP substrate no effect of either colchicine (10^{-3} M) or vinblastine (10^{-4} M) upon pituitary adenylate cyclase activity was seen although prostaglandin E_2 (10^{-6} M) and NaF (10^{-2} M) led to ~2- and 4-fold stimulations of the enzyme under these conditions (not shown). By contrast, both alkaloids led to concentration-dependent inhibitions of pituitary cyclic AMP phosphodiesterase with about half-maximal effects being observed at 10^{-3} M for both colchicine and vinblastine while statistically significant effects were observed at 10^{-5} M colchicine and 10^{-4} M vinblastine (table 4). The effects of the known phosphodiesterase inhibitor, theophylline, are included for comparison (table 4). The colchicine effect was seen equally in the presence and absence of Ca^{2+} (50 μM). Lumicolchicine, produced from colchicine by ultraviolet irradiation at 366 nm, was equipotent with colchicine in these studies (table 4).

Table 2
Effects of calcium deprivation and 2,4-dinitrophenol on colchicine-stimulated growth hormone release (μg NIAMDD-rat GH-RP-1 . h^{-1} . mg wet wt^{-1})

Additions to medium	Period of incubation (h)		*p*
	First	Third	
Colchicine (10^{-3} M) + $CaCl_2$ (2.5×10^{-3} M) + EDTA (0.5×10^{-3} M) [control]	1.92 ± 0.21	3.31 ± 0.30	<0.005
Colchicine (10^{-3} M) + EDTA (0.5×10^{-3} M)	1.64 ± 0.25	2.36 ± 0.27	n.s.
Colchicine (10^{-3} M) [control]	1.48 ± 0.14	2.76 ± 0.37	<0.01
Colchicine (10^{-3} M) + 2,4-dinitrophenol (2.5×10^{-4} M)	1.55 ± 0.27	1.45 ± 0.22	n.s.

Rates of growth hormone release were determined during the first and third hours of incubation in vitro as in section 2; results are expressed as the means ± SEM of 7 obs; n.s., not significant

Table 3
Effects of colchicine, vinblastine, theophylline and prostaglandin E_2 on anterior pituitary cyclic AMP concentrations

Additions to incubation medium	Pituitary cyclic AMP (pmol/mg wet wt)
None [control]	0.47 ± 0.05
Colchicine (10^{-3} M)	1.17 ± 0.16[a]
Vinblastine (10^{-4} M)	0.68 ± 0.06[a]
Theophylline (5×10^{-4} M)	1.00 ± 0.12[a]
Prostaglandin E_2 (10^{-6} M)	1.31 ± 0.23[a]

[a] $p < 0.02$ *vs* control

Pituitary cyclic AMP concentrations were determined as in section 2 at the conclusion of 3 h incubation under the conditions indicated; results are means ± SEM of 9 obs.

4. Discussion

This report documents inhibition of pituitary cyclic AMP phosphodiesterase activity by colchicine and vinblastine with resultant significant increases in cyclic AMP concentrations in pituitary fragments exposed to the agents during incubation in vitro. In the case of colchicine, inhibition of the enzyme was independent of $[Ca^{2+}]$ suggesting that the effect was independent of calmodulin. The activity of lumicolchicine indicates that microtubular disruption is not required for enzyme inhibition by colchicine to be observed.

Colchicine and vinblastine exerted paradoxical stimulatory effects on secretory tissues including adrenal tumor cells in culture [13] and the glucagon-secreting cells of the pancreatic islet [14]. These paradoxical effects of the alkaloids seen in other secretory tissues may be due to alterations in cyclic AMP metabolism. Studies are in progress to evaluate this possibility and to elucidate the nature of the enzyme inhibition observed.

Other examples of colchicine effects not clearly dependent upon microtubular disaggregation are provided by inhibitions of amino acid [15] and nucleoside transport [16] into various cell types by the alkaloid. This report re-emphasises the fact that care should be taken in interpreting data obtained employing colchicine and vinblastine, since their specificity as inhibitors of microtubular function is less absolute than is often assumed.

Table 4
Effects of colchicine, vinblastine and theophylline on anterior pituitary cyclic AMP phosphodiesterase

Additions to reaction mixture	Conc. (M)	Cyclic AMP hydrolysed (pmol . mg wet wt^{-1} . min^{-1})	*p*
None [control]	–	9.51 ± 0.29	–
Colchicine	10^{-5}	8.25 ± 0.35	<0.02
	10^{-4}	7.05 ± 0.33	<0.001
	10^{-3}	4.71 ± 0.25	<0.001
Lumicolchicine	10^{-3}	4.47 ± 0.34	<0.001
Vinblastine	10^{-5}	8.90 ± 0.90	n.s.
	10^{-4}	6.91 ± 0.34	<0.001
	10^{-3}	4.61 ± 0.72	<0.001
Theophylline	2.5×10^{-4}	5.58 ± 0.41	<0.001
	5.0×10^{-4}	4.47 ± 0.28	<0.001
	10^{-3}	2.83 ± 0.55	<0.001

Enzyme activity was determined at a substrate concentration of 10^{-6} M as in section 2; results are the means ± SEM of 6 obs.; n.s., not significant

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