

## The Effect of Colchicine on the Release of Rat Anterior Pituitary Hormones *in Vitro*<sup>1</sup> (37184)

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The drug colchicine has been widely used to study the role of microtubules in cell secretions. It has been shown to bind to a subunit of the microtubules and disaggregate them (1). Colchicine inhibits insulin secretion from the islets of Langerhans (2), histamine secretion from mast cells (3), thyroxine and iodine secretion from the thyroid (4) and catecholamine secretion from the adrenal medulla (5).

In the present experiments we studied the effect of colchicine on the release of the anterior pituitary hormones, luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH) and growth hormone (GH) under both basal and stimulated conditions *in vitro*. Stimulation was accomplished by elevating medium potassium concentration and by the addition of a partially purified extract of sheep hypothalamus.

**Material and Methods.** Male Simonsen rats, weighing 200–250 g, were killed by decapitation, and their pituitaries were halved and randomized in 25 ml Erlenmeyer flasks containing 2 ml of Krebs–Ringer bicarbonate buffer medium with 0.1% glucose (KRBG). Each flask contained 2.5 glands.

The pituitaries were preincubated for 30 min and then transferred to the appropriate test medium. They were then incubated for 3 hr in a Dubnoff metabolic shaker at 37° in an atmosphere of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. In two experiments the glands were prein-

cubated for 2 hr in the presence of colchicine at  $1 \times 10^{-3}$  M, prior to the standard incubation period described above. At the end of each incubation, the medium was removed, centrifuged and the content of LH, FSH, TSH, and GH was measured in the supernatant using specific radioimmunoassays (RIA).<sup>3</sup>

FSH was assayed using the RIA kit supplied by NIAMD. Results were expressed in terms of micrograms per milliliter of FSH NIAMD RP-1 rat standard. LH was measured by the method of Niswender *et al.* (6), using purified LH for radioiodination kindly supplied by Dr. L. E. Reichert. Results were expressed in terms of micrograms per milliliter of NIH LH-S-1. TSH was assayed using antisera generously supplied by Dr. J. F. Wilber (7). The TSH values were expressed in terms of micrograms per milliliter of a purified murine tumor material supplied by Dr. P. Condliff. GH was measured using NIAMD antisera by a method suggested by Dr. G. T. Peake (personal communication). Results were expressed in terms of micrograms per milliliter of NIAMD-Rat-GH-RP-1.

Colchicine was obtained from Calbiochem in Los Angeles, CA (Grade A). The concentrations used in the incubation media ranged from  $1 \times 10^{-3}$  M to  $1 \times 10^{-8}$  M.

A partially purified ovine hypothalamic extract (HE), containing LH-releasing factor (LRF), follicle-stimulating-hormone-re-

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leasing factor (FRF) and thyroid-stimulating-hormone-releasing factor (TRF) activity, was used as a physiological stimulant. The extract was prepared by dilute acetic acid extraction and was purified by ultrafiltration and Sephadex gel filtration. Potassium stimulation of hormone release was accomplished by replacing sodium chloride with an equivalent concentration of potassium chloride. The potassium concentration ranged from 5 to 60 mEq/liter.

In each experiment means and standard errors were determined by pooling the results from all flasks having identical treatments. Levels of significance were determined by application of Student's *t* test.

**Results.** Colchicine significantly increased the basal release of GH ( $p < 0.01$  in all experiments) (Tables I and II). The drug either had no effect or very slightly decreased the basal release of LH, FSH and TSH. This decrease was only significant in one experiment with FSH.

In every experiment the hypothalamic extract significantly increased the rate of release of LH, FSH and TSH. Significant GRF activity was only demonstrable in one ex-

periment (Table I, Expt 2) ( $p < 0.01$ ). Colchicine significantly enhanced the action of LRF and FRF in releasing LH and FSH (Table I). The effective dose of colchicine for LRF potentiation was from  $10^{-6}$  to  $10^{-3}$  *M*, while the potentiation for FRF was only significant at  $10^{-3}$  *M*. Colchicine also appeared to enhance the action of TRF in releasing TSH, although this was never significant statistically. In the single experiment in which GRF activity was demonstrated, colchicine at  $1 \times 10^{-6}$  *M* significantly potentiated ( $p < 0.05$ ) the stimulated release of GH (Table I, Expt 2).

In every experiment, increasing the potassium concentration in the medium to 25 or 35 mM significantly increased the release of LH, FSH, TSH and GH. The addition of colchicine at concentrations ranging from  $10^{-6}$  to  $10^{-3}$  *M* to the potassium-stimulated pituitary cell appeared to slightly increase the release of LH, FSH and TSH but GH only in one of three cases. These increases were not significant (Table II).

To further clarify the effect of colchicine on pituitary hormone release, we performed a series of experiments in which the glands

TABLE I. Effect of Colchicine on Basal and HE-Stimulated Hormone Release *in Vitro* [Values Are Amount of Hormone Released ( $\mu\text{g/ml/3 hr}$ )].

| Treatment             | No. <sup>a</sup> | GH               | LH                | FSH              | TSH            |
|-----------------------|------------------|------------------|-------------------|------------------|----------------|
| Expt 1                |                  |                  |                   |                  |                |
| Control               | 8                | —                | $0.58 \pm 0.09$   | $10.1 \pm 1.8$   | $3.5 \pm 0.4$  |
| Colch, $10^{-3}$      | 3                | —                | $0.47 \pm 0.07$   | $8.9 \pm 0.7$    | $2.7 \pm 0.6$  |
| Colch, $10^{-6}$      | 5                | —                | $0.65 \pm 0.14$   | $10.1 \pm 0.3$   | $3.1 \pm 0.4$  |
| HE, 0.87 mg/ml        | 8                | —                | $1.16 \pm 0.11$   | $15.3 \pm 0.4$   | $12.6 \pm 1.1$ |
| HE + colch, $10^{-3}$ | 5                | —                | $1.21 \pm 0.09$   | $15.2 \pm 1.0$   | $12.3 \pm 0.8$ |
| HE + colch, $10^{-6}$ | 5                | —                | $1.41 \pm 0.25$   | $15.5 \pm 1.6$   | $13.7 \pm 0.8$ |
| Expt 2                |                  |                  |                   |                  |                |
| Control               | 8                | $19.6 \pm 2.5^b$ | $0.55 \pm 0.11$   | $7.1 \pm 1.2$    | $3.8 \pm 0.5$  |
| Colch, $10^{-6}$      | 8                | $31.2 \pm 2.8^d$ | $0.39 \pm 0.04$   | $5.8 \pm 0.7$    | $3.8 \pm 0.4$  |
| HE, 0.87 mg/ml        | 8                | $27.8 \pm 1.3$   | $1.14 \pm 0.06$   | $11.2 \pm 0.7$   | $28.2 \pm 3.5$ |
| HE + colch, $10^{-6}$ | 8                | $35.4 \pm 2.6^c$ | $1.54 \pm 0.13^c$ | $11.2 \pm 0.6$   | $34.5 \pm 1.7$ |
| Expt 3                |                  |                  |                   |                  |                |
| Control               | 4                | $42.5 \pm 2.8$   | $0.38 \pm 0.02$   | $6.7 \pm 0.1$    | $2.8 \pm 0.1$  |
| Colch, $10^{-3}$      | 4                | $80.8 \pm 7.1^d$ | $0.33 \pm 0.04$   | $5.6 \pm 0.4^c$  | $2.6 \pm 0.1$  |
| HE, 1.73 mg/ml        | 4                | $38.9 \pm 2.5$   | $1.04 \pm 0.05$   | $13.5 \pm 0.1$   | $15.0 \pm 1.1$ |
| HE + colch, $10^{-3}$ | 4                | $78.3 \pm 5.2^d$ | $1.46 \pm 0.13^c$ | $18.1 \pm 1.4^c$ | $18.1 \pm 1.6$ |

<sup>a</sup> Number of flasks per group.

<sup>b</sup> Mean  $\pm$  standard error.

<sup>c</sup> Vs preceding flask:  $p < .05$ ; <sup>d</sup>  $p < .01$ .

TABLE II. Effect of Colchicine on K<sup>+</sup>-Stimulated Release of Pituitary Hormones *in Vitro* [Values Are Amount of Hormone Released ( $\mu\text{g}/3\text{ hr}$ )].

| Treatment                          | No. <sup>a</sup> | GH                            | LH              | FSH            | TSH            |
|------------------------------------|------------------|-------------------------------|-----------------|----------------|----------------|
| Expt 1                             |                  |                               |                 |                |                |
| Control                            | 4                | 40.9 $\pm$ 0.6 <sup>b</sup>   | 0.23 $\pm$ 0.03 | 3.7 $\pm$ 0.5  | 2.6 $\pm$ 0.4  |
| K, 35 mM                           | 4                | 261.7 $\pm$ 18.6              | 0.81 $\pm$ 0.04 | 13.0 $\pm$ 1.2 | 30.6 $\pm$ 5.7 |
| K, 35 mM + colch, 10 <sup>-6</sup> | 4                | 264.3 $\pm$ 12.6              | 0.75 $\pm$ 0.07 | 12.7 $\pm$ 1.6 | 24.8 $\pm$ 4.2 |
| K, 35 mM + colch, 10 <sup>-5</sup> | 4                | 251.5 $\pm$ 12.4              | 0.92 $\pm$ 0.07 | 14.0 $\pm$ 0.7 | 26.4 $\pm$ 3.9 |
| Expt 2                             |                  |                               |                 |                |                |
| Control                            | 6                | 39.3 $\pm$ 6.0                | 0.29 $\pm$ 0.04 | 4.9 $\pm$ 0.2  | 1.1 $\pm$ 0.1  |
| K, 25 mM                           | 6                | 162.5 $\pm$ 6.8               | 0.52 $\pm$ 0.02 | 8.4 $\pm$ 0.4  | 4.4 $\pm$ 0.5  |
| K, 25 mM + colch, 10 <sup>-5</sup> | 6                | 151.3 $\pm$ 10.1              | 0.54 $\pm$ 0.03 | 9.1 $\pm$ 0.7  | 4.8 $\pm$ 0.3  |
| Expt 3                             |                  |                               |                 |                |                |
| Control                            | 4                | 178.3 $\pm$ 7.9               | 0.15 $\pm$ 0.01 | 4.9 $\pm$ 0.5  |                |
| Colch, 10 <sup>-3</sup>            | 4                | 259.8 $\pm$ 20.1 <sup>c</sup> | 0.13 $\pm$ 0.03 | 4.2 $\pm$ 0.2  |                |
| K, 25 mM                           | 4                | 423.0 $\pm$ 16.0              | 0.23 $\pm$ 0.02 | 9.1 $\pm$ 0.5  |                |
| K, 25 mM + colch, 10 <sup>-3</sup> | 4                | 462.3 $\pm$ 13.2              | 0.31 $\pm$ 0.05 | 10.1 $\pm$ 0.8 |                |

<sup>a</sup> Number of flasks per treatment.<sup>b</sup> Mean  $\pm$  standard error.<sup>c</sup>  $p < .01$  vs preceding flask.

were preincubated in colchicine at 10<sup>-3</sup> M prior to the standard incubation period (Table III). Under these conditions, colchicine had no significant effect on the basal release of LH or FSH while the potentiating effect of colchicine on LRF and FRF activity was magnified. In this same system, colchicine significantly enhanced the release of LH and FSH when the glands were stimulated with 50 mM of potassium (Table III). We are unable to explain the decreased basal release of LH in these two experiments, although this has been consistent in recent months.

*Discussion.* Although colchicine has been shown to inhibit the release of hormones from a number of endocrine organs, we find that it enhances the stimulated release of the various pituitary hormones studied. In the case of GH, the basal release was increased by colchicine; whereas, in the case of FSH and LH, the stimulated release brought about by HE or 50 mM of potassium was potentiated by the drug. Enhancement of potassium-induced LH and FSH release by colchicine was only significant when the glands were preincubated for 2 hr with the drug. There was no

TABLE III. Effect of Colchicine on Basal, HE and Potassium Stimulated Hormone Release After a 2 hr Preincubation Period in Colchicine *in Vitro* (Values are Given in  $\mu\text{g}$  of Hormone Released/ml/3 hr).

| Treatment                 |  | No. <sup>a</sup> | LH                            | FSH                           |
|---------------------------|--|------------------|-------------------------------|-------------------------------|
| Preincubation             | Incubation                                       |                  |                               |                               |
| Control media             | Control media                                    | 4                | 0.11 $\pm$ 0.007 <sup>b</sup> | 5.88 $\pm$ 0.48               |
| Colch, 10 <sup>-3</sup> M | Colch, 10 <sup>-3</sup> M                        | 4                | 0.13 $\pm$ 0.014              | 6.63 $\pm$ 0.81               |
| Control media             | HE, 1.6 mg/ml                                    | 4                | 0.31 $\pm$ 0.020              | 11.55 $\pm$ 0.80              |
| Colch, 10 <sup>-3</sup> M | HE + Colch, 10 <sup>-3</sup> M                   | 4                | 0.44 $\pm$ 0.036 <sup>c</sup> | 15.38 $\pm$ 0.54 <sup>a</sup> |
| Control media             | Control media                                    | 4                | 0.18 $\pm$ 0.022              | 6.53 $\pm$ 0.18               |
|                           | K <sup>+</sup> , 55 mM                           | 4                | 0.52 $\pm$ 0.030              | 18.13 $\pm$ 0.61              |
| Colch, 10 <sup>-3</sup> M | K <sup>+</sup> , 55 mM + Colch, 10 <sup>-3</sup> | 4                | 0.82 $\pm$ 0.033 <sup>d</sup> | 27.72 $\pm$ 1.04 <sup>a</sup> |

<sup>a</sup> Number of flasks per group.<sup>b</sup> Mean  $\pm$  standard error.<sup>c</sup> Vs preceding flask:  $p < .05$ ; <sup>d</sup>  $p < .01$ .

significant effect of colchicine on TRF-stimulated TSH release.

While this work was in progress, several other reports of the effect of colchicine on the release of anterior pituitary hormones appeared. Lockhart-Ewart and Taylor (8) observed enhancement of basal and cyclic-AMP-stimulated GH release from rat pituitaries. Temple *et al.* (9) were unable to demonstrate an effect of colchicine on synthetic-TRF-stimulated TSH release *in vitro*.

In their studies on control of GH secretion, Schofield and Cole (10) and Cooper, McPherson and Schofield (11) used colchicine in a system utilizing a 2 hr preincubation period in the presence of the drug, followed by a 30 to 45 min incubation period of ox pituitary slices. In this system they observed an increased basal release of growth hormone from pituitaries incubated in  $5 \times 10^{-3}$  M colchicine, but not at  $1 \times 10^{-4}$  or  $1 \times 10^{-5}$  M (10, 11). In contrast they found that  $5 \times 10^{-3}$  M colchicine inhibited the release of GH stimulated with 71 mM of potassium (10). Although there is an apparent discrepancy from our data, this may be attributable to differences in experimental design. Kraicer and Milligan (12) also reported that  $1 \times 10^{-5}$  M colchicine inhibited the release of ACTH induced by a five-fold elevation of the medium potassium concentration.

The only other report of a stimulatory effect of colchicine on hormonal release is that of Temple *et al.* (9) who reported that colchicine stimulated the release of corticosteroids from mouse adrenal tumor cells.

Since our data indicate that colchicine enhances the stimulated release of the various pituitary hormones studied, it would appear that microtubules might be involved in regulating the distribution of hormone-containing granules within the pituitary cell. Perhaps they anchor the granules or regulate their movement towards the plasma membrane after their assembly in the Golgi apparatus. When the microtubules are disrupted, more granules accumulate at the surface to become available for stimulated release. Pelletier and

Bornstein (13) have, in fact, observed an accumulation of secretory granules in somatotrophs in tissue culture 6 hr after the addition of colchicine.

*Summary.* 1. Colchicine significantly increased the basal rate of release of growth hormone while it did not effect the basal rate of release of LH, FSH or TSH from anterior pituitaries incubated *in vitro*.

2. Colchicine significantly enhanced the action of LRF and FRF by increasing the rate of release of LH and FSH. This effect was magnified by preincubating the pituitaries in colchicine for 2 hr.

3. Colchicine at  $1 \times 10^{-3}$  M significantly potentiated potassium-stimulated LH and FSH release only when the colchicine-treated glands were preincubated with the drug for 2 hr prior to incubation.

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