is eventually prevented due to an interruption of cellular proliferations, which is caused by a strong reduction in DNA contents of granulomatous tissue in rats treated with hydroxyurea.

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INHIBITIONS BY COLCHICINE, VINBLASTINE AND CYTOCHALASIN-B OF THE STIMULATORY EFFECT OF THE CYTOPLASMIC FRACTION ON CATECHOLAMINE RELEASE FROM ADRENOMEDULLARY GRANULES

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Microtubules and microfilaments have been regarded as being involved in release of hormones and amines, since agents, such as colchicine and vinblastine, and cytochalasin-B, which disrupt microtubules and microfilaments, respectively, inhibit the release of hormones or amines from cells (1-11). Stimulation of the release of catecholamine from perfused adrenal gland by acetylcholine or nicotine is also inhibited by colchicine, vinblastine or cytochalasin-B (12-15).

In our previous studies on the intracellular mechanism of catecholamine release, using isolated adrenal medullary granules, it was demonstrated that the release of catecholamine is stimulated by ATP·Mg⁺⁺ in the presence of a low concentration of Ca⁺⁺ (<10⁻⁵ M) (16, 17) and that a cytoplasmic fraction of adrenal medulla (supernatant obtained by centrifugation at 105,000 × g for 2 hr) accelerates ATP·Mg⁺⁺ stimulated catecholamine release from the granules (18).

In the present work, the effects of colchicine, vinblastine and cytochalasin-B on the stimulatory effect of the cytoplasmic fraction on catecholamine release were examined, to determine whether microtubules and microfilaments-like components are involved in the stimulatory effect of the cytoplasmic fraction. Catecholamine storage granules and the cytoplasmic fraction from bovine adrenal medulla were prepared as described previously (18). Incubations were carried out as described in the footnote of Fig. 1.

Results are summarized in Fig. 1. During the incubation period, 18.2 ± 3.6% (mean ±

FIG. 1. Effects of colchicine, vinblastine and cytochalasin-B on the release of catecholamine from adrenal medullary granules.

Incubations were carried out at 37°C for 5 min in centrifuge tubes without shaking. The medium contained 150 mM KCl and 10 mM Tris-Maleate buffer (pH 7.0) with or without 2 mM ATP-1 mM MgCl₂ and the cytoplasmic fraction (1.5 mg/ml) in a final volume of 3 ml. After pre-incubation for 5 min, the reaction was started by adding the granules (about 2 mg of protein, containing 400–500 µg of catecholamine). The reaction was stopped by adding 4 ml of ice cold 150 mM KCl containing 10 mM Tris-Maleate buffer (pH 7.0). The tubes were then chilled in ice and centrifuged at 20,000 × g for 10 min. Catecholamine in the precipitate and supernatant was extracted with 0.4 N perchloric acid and determined fluorimetrically by the ethylenediamine condensation method (19).

Columns, and bars represent means ± S.D. of the releases in 4 to 12 experiments, expressed as percentages of the total catecholamine.

□; spontaneous release, ▨; release with ATP·Mg⁺⁺, ■; release with ATP·Mg⁺⁺ and the cytoplasmic fraction. * Cytochalasin-B was dissolved in 0.25% (v/v) dimethylsulfoxide

S.D.; n=12) of the total catecholamine was released spontaneously from the granules. Release of catecholamine increased to 40.5 ± 5.8% (mean ± S.D.; n=12) on addition of ATP and Mg⁺⁺, and to 73.7 ± 11.2% (mean ± S.D.; n=12) on addition of the cytoplasmic fraction with ATP and Mg⁺⁺, thus confirming previous results (18). Addition of the cytoplasmic fraction in the absence of ATP and Mg⁺⁺ did not increase the release of catecholamine.

Colchicine at concentrations of 10⁻⁴ M and 10⁻³ M was found to inhibit the stimulatory effect of the cytoplasmic fraction on catecholamine release by 30% and 56%, respectively. A low concentration of colchicine (10⁻⁵ M) was only partially inhibitory. Colchicine at a concentration of 10⁻³ M had no effect on the spontaneous release of catecholamine, but slightly inhibited ATP·Mg⁺⁺ stimulated catecholamine release. Colchicine was more inhibitory when pre-incubated with the cytoplasmic fraction, that is when the cytoplasmic fraction was pre-incubated with 10⁻⁴ M colchicine for 2 hr, 50% inhibition was observed (results not shown).

Vinblastine was also found to inhibit the stimulatory effect of the cytoplasmic fraction on catecholamine release. It was inhibitory at lower concentrations than colchicine, and concentrations of 10^{-6} M, 10^{-5} M and 10^{-4} M caused 30%, 40% and 52% inhibition of the effect of the cytoplasmic fraction, respectively. Concentrations of more than 10^{-5} M significantly increased the spontaneous release of catecholamine, but had little effect on ATP-Mg⁺⁺ stimulated catecholamine release.

Cytochalasin-B also inhibited the stimulatory effect of the cytoplasmic fraction. It was effective at concentrations similar to those used for vinblastine, and concentrations of 3×10^{-6} M and 3×10^{-5} M inhibited the effect of the cytoplasmic fraction by 34% and 52%, respectively. Spontaneous release appeared to be stimulated much to the same degree by 3×10^{-5} M cytochalasin-B and 10^{-5} M vinblastine.

From the above results, it is suggested that the cytoplasmic fraction contains elements which are affected by colchicine, vinblastine and cytochalasin-B and that these elements (possibly microtubules and microfilaments-like elements) may be involved in the stimulatory effect of the cytoplasmic fraction on the release of catecholamines from adrenomedullary granules.

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