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Inhibition of Release of Dopamine-β-Hydroxylase and Norepinephrine from Sympathetic Nerves by Colchicine, Vinblastine, or Cytochalasin-B

(hypogastric nerve stimulation/exocytosis/microtubules/microfilaments/guinea pig)

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ABSTRACT **Stimulation of the hypogastric nerve to the guinea pig vas deferens in the presence of phenoxybenzamine produced an enhanced release of both dopamine-β-hydroxylase (EC 1.14.2.1) and norepinephrine. Addition of colchicine, vinblastine, or cytochalasin-B to the incubation medium caused an almost complete inhibition of release of both the amine and the enzyme. Colchicine and cytochalasin-B produced a blocking effect but did not, however, modify the ratio of dopamine-β-hydroxylase to norepinephrine recovered in the bath fluid. The findings indicate that norepinephrine and dopamine-β-hydroxylase are released from sympathetic nerve terminals by exocytosis and that this process is dependent upon the integrity of both microtubules and microfilaments.**

Investigations of the physiologic function of two widely distributed cell components, the microtubules and microfilaments, have been facilitated by the use of the alkaloids colchicine and vinblastine, which bind to the microtubules (1, 2), and of cytochalasin-B, a fungal metabolite that causes the disappearance of the microfilaments (3). The microtubules have been implicated in release of various intracellular stored products, such as insulin from the beta-cells of the pancreas (4), iodine-131 from isolated thyroid glands (induced by thyroid-stimulating hormone) (5), histamine from mast cells (6), and catecholamine from the adrenal medulla (7). Release of iodine-131 from the thyroid gland has also been shown to be inhibited by cytochalasin-B (8).

It has been shown (9) that electrical stimulation of the hypogastric nerves to the vas deferens of guinea pig results in a proportional release of both the neurotransmitter norepinephrine and dopamine-β-hydroxylase (EC 1.14.2.1), an enzyme present in the norepinephrine storage vesicles in both bound and free forms (10, 11). Observation of release of norepinephrine and dopamine-β-hydroxylase from the adrenal gland that is stimulated by acetylcholine (11) suggests that exocytosis is the process by which norepinephrine release occurs. Because of the similarity of the ratios of norepinephrine to dopamine-β-hydroxylase in tissue and in the amounts of norepinephrine and dopamine-β-hydroxylase released (9, 12), dopamine-β-hyroxylase may be considered a reliable index of exocytosis.

In the present study we show that agents altering the microtubules and microfilaments block the nerve stimulation-induced release of dopamine-β-hydroxylase and norepinephrine by exocytosis at the sympathetic nerve endings of the vas deferens.

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MATERIALS AND METHODS

Preparation of Organs. The hypogastric nerve–vas deferens preparations were isolated and prepared for stimulation as described (9, 12). The bath fluid was changed five times and then replaced by fresh medium (Krebs–Ringer, without bicarbonate, pH 7.2–7.4) containing 0.25% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). After 10 min, the medium was replaced with 6 ml of medium containing albumin and phenoxybenzamine hydrochloride (50 μM). The use of phenoxybenzamine is necessary to block reuptake of norepinephrine (9, 12). The preparations were allowed to equilibrate for 5 min before the stimulation period.

The hypogastric nerve was stimulated electrically (5–7 V, 25 Hz, 5 msec) for 30 sec of each minute for 60 min. When agents other than phenoxybenzamine were used, they were added to the albumin-containing medium both for the 10 min before addition of the phenoxybenzamine-containing medium and for the 5-min period before stimulation, in the presence of phenoxybenzamine.

Assay of Dopamine-β-Hydroxylase and Norepinephrine. Dopamine-β-hydroxylase and norepinephrine in the incubation medium were determined as described (9, 12).

Drugs. Phenoxybenzamine hydrochloride was kindly supplied by the Smith, Kline and French Laboratories (Philadelphia, Pa.). Colchicine was purchased from Calbiochem (Los Angeles, Calif.), and vinblastine sulfate (Velban) was obtained from Eli Lilly and Company (Indianapolis, Ind.). Cytochalasin-B was the kind gift of the Imperial Chemical Industries, Inc. (Wereside Alderley Park, Wacclesfield Cheshire, England). It was dissolved in dimethylsulfoxide and added to the incubation medium, to a final concentration of 0.1% dimethylsulfoxide.

RESULTS

Effect of colchicine on release of dopamine-β-hydroxylase and norepinephrine

Electrical stimulation of the hypogastric nerve for 1 hr caused a marked release of norepinephrine and dopamine-β-hydroxylase into the bath fluid. The ratio of dopamine-β-hydroxylase to norepinephrine was identical, whether release was spontaneous or electrically induced (Fig. 1). 1 mM Colchicine almost completely inhibited the release of both dopamine-β-hydroxylase and norepinephrine after stimulation. Lower doses of the alkaloid (0.5 and 0.75 mM) are only partially inhibitory. Colchicine had no effect on the spontaneous release of either

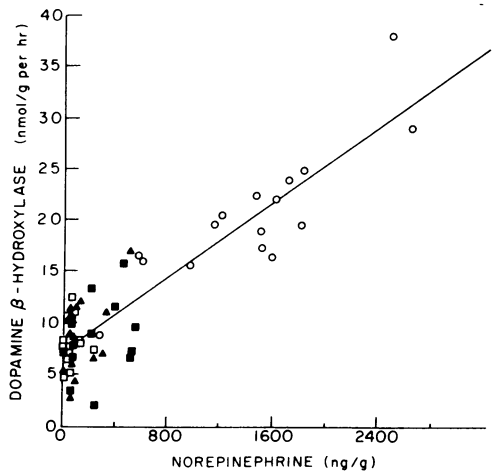

FIG. 1. Proportionality of release of dopamine-β-hydroxylase and norepinephrine from the vas deferens. The dopamine-β-hydroxylase activity present in the incubation medium is plotted against the concentration of norepinephrine released into the bath: □ Unstimulated controls, ○ 60-min stimulation controls, ■ stimulated colchicine-treated organs, ▲ stimulated cytochalasin-B-treated organs. $Y = 7.1 + 0.0094\ X$; correlation coefficient = 0.893; significance (P) <0.001.

dopamine-β-hydroxylase or norepinephrine (Fig. 2), nor did it alter the ratio of dopamine-β-hydroxylase to norepinephrine released (Fig. 1).

Effect of vinblastine on release of dopamine-β-hydroxylase

0.1 mM Vinblastine almost completely inhibited the release of dopamine-β-hydroxylase that was produced by stimulation. In unstimulated organs, vinblastine caused a significant increase of spontaneous release of dopamine-β-hydroxylase (Fig. 3).

Effect of cytochalasin-B on release of dopamine-β-hydroxylase and norepinephrine

Cytochalasin-B (3 μg/ml) almost completely blocked the release of both dopamine-β-hydroxylase and norepinephrine in stimulated organs. It had little effect on the spontaneous discharge of either the neurotransmitter or the enzyme (Fig. 4). Cytochalasin-B did not change the ratio of released dopamine-β-hydroxylase to norepinephrine (Fig. 1).

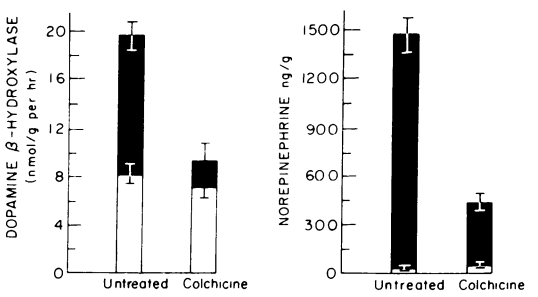

FIG. 2. Effect of 1 mM colchicine on dopamine-β-hydroxylase and norepinephrine release by guinea pig vasa deferentia. The *lower open bars* represent bath concentration from unstimulated organs. *Total bars* represent total activity recovered after 1 hr of hypogastric nerve stimulation. *Shaded areas* represent the stimulated release of dopamine-β-hydroxylase and norepinephrine activity. *Each bar* represents the mean (±SE) for at least eight preparations. $P < 0.001$ for untreated, stimulated organs versus colchicine-treated, stimulated organs both for dopamine-β-hydroxylase and norepinephrine.

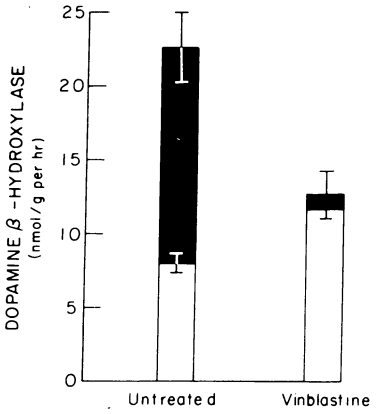

FIG. 3. Effect of 1 mM vinblastine on release of dopamine-β-hydroxylase. The *lower bars* represent activity of dopamine-β-hydroxylase in incubation medium with unstimulated organs. *Shaded areas* represent the stimulation-induced dopamine-β-hydroxylase activity. *Each bar* is the mean (±SE) for at least eight preparations. $P < 0.001$ for untreated, stimulated organs versus vinblastine-treated, stimulated organs.

DISCUSSION

Both electronmicroscopic (13–15) and biochemical evidence (9, 11, 12) indicate that the adrenergic nerve discharges the neurotransmitter by a process of exocytosis. Exocytosis presumably involves a fusion of norepinephrine storage-vesicle membrane with the neuronal membrane, followed by an extrusion of both the neurotransmitter and the soluble contents of the vesicles, including the high-molecular-weight protein (300,000), dopamine-β-hydroxylase. Thus, release is coupled with depolarization (9, 12, 16) and is facilitated by calcium ions as well as by phenoxybenzamine (12). In contrast to slower discharge of hormones such as insulin and thyroxin, release of neurotransmitters occurs in a fraction of a second.

Our present study indicates that the integrity of microtubule proteins is critical for depolarization-induced exocyto-

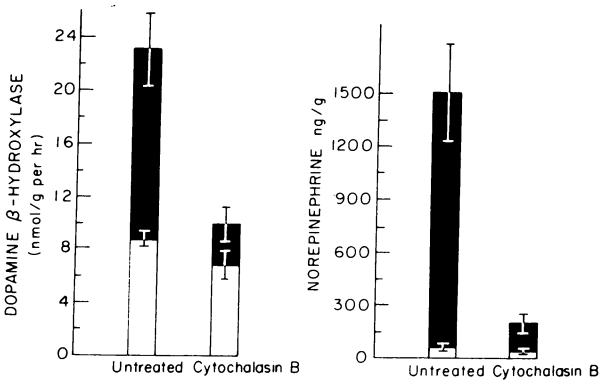

FIG. 4. Effect of cytochalasin-B (3 μg/ml) on release of dopamine-β-hydroxylase and norepinephrine. The *lower bars* represent dopamine-β-hydroxylase activity and norepinephrine in incubation medium with unstimulated organs. *Shaded areas* represent stimulation-induced activities. *Each bar* is the mean (±SE) for at least eight preparations. $P < 0.001$ for untreated, stimulated organs versus cytochalasin-B-treated preparations.

sis since it is inhibited by both colchicine and vinblastine, two agents known to disaggregate the tubular proteins by binding at two different subunits of the protein (17). Microtubular proteins have also been shown to be involved in the fast proximodistal flow of norepinephrine (18, 19) from the cell body to the nerve terminals. Since microtubular proteins are believed to function as cytoskeleton, it is possible that they may serve to orient the vesicles to the sites of the neuronal membrane from which release is to occur.

Cytochalasin-B, a compound that disrupts the function of the contractile-filament system in cells (20), inhibits the release of both dopamine-β-hydroxylase and norepinephrine seen during stimulation of the hypogastric nerve. It has been shown that in cells other than muscle, microfilaments are activated by calcium (20). Release of dopamine-β-hydroxylase and norepinephrine by exocytosis has also been shown to be activated by calcium (12). Thus, it seems likely that the rapid release of neurotransmitter may involve a contractile mechanism similar to that occurring in muscle.

Recent results (unpublished) from this laboratory show that electrical stimulation of the sympathetic nerve to isolated perfused cat spleen is accompanied by a stoichiometric release of both dopamine-β-hydroxylase and norepinephrine. The release is also enhanced by phenoxybenzamine and inhibited by colchicine.

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