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Enhanced Release of Dopamine β-Hydroxylase and Norepinephrine from Sympathetic Nerves by Dibutyryl Cyclic Adenosine 3′, 5′-Monophosphate and Theophylline

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SUMMARY

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Stimulation of the hypogastric nerve to the guinea pig vas deferens *in vitro* in the presence of a normal extracellular calcium concentration produced a proportional release of dopamine β-hydroxylase (EC 1.14.2.1) and norepinephrine. Both dibutyryl cyclic AMP (0.1 mM) and theophylline (1 mM) enhanced stimulation-induced release of dopamine β-hydroxylase and norepinephrine, with dibutyryl cyclic AMP also increasing spontaneous release. In a calcium-free medium the stimulation-induced release of dopamine β-hydroxylase and norepinephrine was completely blocked. However, stimulation-induced release did occur when either dibutyryl cyclic AMP (0.1 mM) or theophylline (1 mM) was added to the calcium-free medium. As in the medium containing a normal calcium concentration, dibutyryl cyclic AMP enhanced spontaneous release of dopamine β-hydroxylase and norepinephrine. The ratio of dopamine β-hydroxylase to norepinephrine released was identical whether release was spontaneous or electrically induced and whether or not dibutyryl cyclic AMP or theophylline was present. Thus dibutyryl cyclic AMP and theophylline do not require extracellular calcium to cause the release of neurotransmitter by exocytosis at sympathetic nerve endings. These results suggest that cyclic 3′,5′-AMP may have a role in release, acting either in parallel with calcium or indirectly by mobilizing intracellular, bound calcium.

INTRODUCTION

A physiological role for cyclic 3′,5′-AMP and calcium in excitation-secretion coupling has been demonstrated in a variety of neural and endocrine tissues. Following specific stimuli, cyclic 3′,5′-AMP has been found to accumulate in tissues during release of thyrocalcitonin (1), insulin (2), salivary secretions (3, 4), and gastric HCl (5). Also, dibutyryl cyclic AMP and the methylxanthines, which inhibit phosphodiesterase and thereby elevate levels of cyclic AMP (6), enhance acetylcholine release at the neuromuscular junction (7) and cause release of catecholamines from heart-lung preparations (8), the brain (9), and the adrenal medulla (10). Release of neurotransmitters from both cholinergic nerve endings (11–13) and the adrenal medulla (14, 15) is associated with an influx of calcium. Furthermore, stimulus-induced release of norepinephrine from sympathetic nerves has been found to vary directly with extracellular calcium concentration over a wide range (16). Nevertheless,

the precise interrelationship between calcium and cyclic AMP in stimulus-secretion coupling remains controversial (17, 18).

Recent studies have shown that electrical stimulation of the hypogastric nerve of the isolated guinea pig vas deferens results in a proportional release of both the neurotransmitter norepinephrine and dopamine β-hydroxylase (EC 1.14.2.1) (19), an enzyme present in norepinephrine storage vesicles in both bound and free forms (20, 21). Because the ratio of catecholamine to dopamine β-hydroxylase in the soluble form in tissue approximates the ratio of catecholamine and enzyme released (19, 20), it has been suggested that exocytosis is the mechanism by which norepinephrine secretion occurs from the adrenal medulla (20) and sympathetic nerves (22, 23, 19) and that dopamine β-hydroxylase release is an index of exocytosis.

In the present study we have examined the effects of dibutyryl cyclic AMP and theophylline on the release of dopamine β-hydroxylase and norepinephrine from the guinea pig vas deferens before and during stimulation of the hypogastric nerve.

METHODS

Preparation of organs. The hypogastric nerve–vas deferens preparations were isolated and prepared for stimulation as previously described (19, 24, 25). The preparations were washed five times with Krebs-Ringer solution without bicarbonate, pH 7.2–7.4, and then changed to Krebs-Ringer solution containing 0.25% bovine serum albumin (Sigma Chemical Company). The Krebs-Ringer solution had the following composition per liter: NaCl, 8.06 g; KCl, 0.35 g; $CaCl_2 \cdot 2H_2O$, 0.3 g; $MgSO_4 \cdot 7H_2O$, 294 mg; KH_2PO_4, 162 mg; glucose, 2.07 g. The solution was constantly aerated with 5% CO_2 and 95% O_2. After 15 min the medium was replaced by Krebs-Ringer solution containing 0.25% BSA and phenoxybenzamine HCl (50 μM). Phenoxybenzamine was used to block reuptake of norepinephrine. After 5 min stimulation of the hypogastric nerves was begun (30 sec of every 1 min for 60 min, 5–7 V, 25 Hz, 5 msec). When drugs were used, they were first incubated for 15 min in Krebs-Ringer solution and 0.25% BSA, pH 7.2–7.4. Incubation was continued for another 5 min after the solution had been replaced with Krebs-Ringer solution containing 0.25% BSA and 50 μM phenoxybenzamine HCl, and the drug and then the nerves were stimulated as described above. For the experiments in which a calcium-free medium was used, $CaCl_2 \cdot 2H_2O$ was omitted from the preparation of the various Krebs-Ringer solutions.

Assay of norepinephrine and dopamine β-hydroxylase. Norepinephrine was assayed fluorometrically in the bath fluid and vas deferens homogenates by the trihydroxyindole method, as previously described (19, 25). Dopamine β-hydroxylase was assayed in the bath medium and vas deferens homogenates by the method of Molinoff *et al.* (26), as previously described (19, 25). Optimal dopamine β-hydroxylase activity in the bath medium was obtained in the presence of a copper concentration of 20 μM copper sulfate and in the vas deferens homogenates at a copper sulfate concentration of 13 μM. One unit of dopamine β-hydroxylase activity represents the formation of 1 nmole of octopamine per gram per hour.

Drugs. Phenoxybenzamine HCl was obtained from Smith Kline & French Laboratories; dibutyryl cyclic AMP and 3′,5′-cyclic AMP, from Calbiochem; and theophylline, from Nutritional Biochemicals Corporation.

RESULTS

Effects of dibutyryl cyclic AMP and theophylline on release of dopamine β-hydroxylase and norepinephrine in normal medium. Electrical stimulation of the hypogastric nerve for 1 hr resulted in marked release of norepinephrine and dopamine β-hydroxylase into the bath fluid. The release of dopamine β-hydroxylase was proportional to that of norepinephrine. The ratio of dopamine β-hydroxylase to norepinephrine in the bath medium was identical whether release was spontaneous or electrically induced and whether or not theophylline or dibutyryl cyclic AMP was present (Fig. 1). Stimulation of the vas deferens for 1 hr resulted in a 25% decrease in tissue norepinephrine content. The norepinephrine concentration in unstimulated control organs was 8160 ± 280 ng/g while the norepinephrine content of

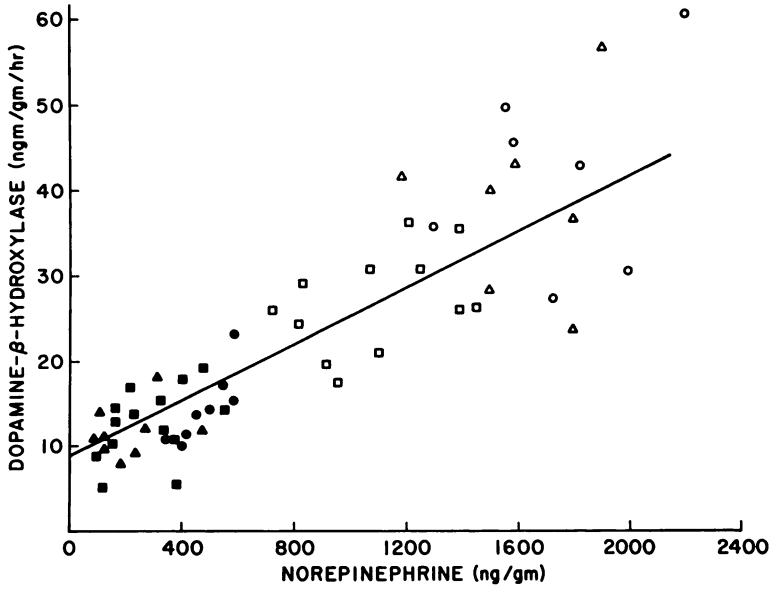

FIG. 1. *Proportionality of release of dopamine β-hydroxylase and norepinephrine from vas deferens*
The dopamine β-hydroxylase activity present in the incubation medium is plotted against the concentration of norepinephrine in the medium: solid symbols denote unstimulated preparations; open symbols, stimulated preparations; squares, no drug treatment; circles, dibutyryl cyclic AMP (0.1 mM); triangles, theophylline (1 mM). $y = 8.92 + 0.0165x$; correlation coefficient = 0.85; significance (p) < 0.001.

the stimulated organs was 6130 ± 260 ng/g ($p < 0.01$). Whole organ dopamine β-hydroxylase activity was not significantly reduced by stimulation, with control organ activity averaging 1920 ± 100 nmoles of octopamine per gram per hour, and mean activity after 1 hr of stimulation, 1800 ± 90 nmoles of octopamine per gram per hour. Neither dibutyryl cyclic AMP nor theophylline had any effect on control tissue levels of norepinephrine and dopamine β-hydroxylase after 1 hr of incubation.

Dibutyryl cyclic AMP (0.1 mM) enhanced spontaneous as well as stimulation-induced release of dopamine β-hydroxylase and norepinephrine (Fig. 2). Neither cyclic AMP (0.3 mM) nor butyric acid (0.1 mM) had any effect on release.

Theophylline (1 mM) enhanced stimulation-induced release of both dopamine β-hydroxylase and norepinephrine, but had no effect on spontaneous release (Fig. 2).

Effects of dibutyryl cyclic AMP and theophylline on release of dopamine β-hydroxylase and norepinephrine in calcium-free medium. In the absence of calcium and drugs there was no stimulation-induced release of dopamine β-hydroxylase or norepinephrine. However, in spite of the absence of calcium, stimulation-induced release did occur in the presence of either dibutyryl cyclic AMP (0.1 mM) or theophylline (1 mM) (Fig. 3). As was found in medium containing a normal concentration of calcium, spontaneous release of dopamine β-hydroxylase and norepinephrine was enhanced by dibutyryl cyclic AMP, but not by theophylline.

DISCUSSION

In a large number of cell systems both cyclic 3′,5′-AMP and calcium appear to play roles in the coupling of cell excitation and secretory release (17). In most systems excitation of the cell is followed by a rise in intracellular cyclic AMP and an influx of extracellular calcium ions. However, the precise interrelationship between cyclic AMP and calcium in excitation-secretion coupling is unclear and may differ in tissues such as the parotid gland and adrenal cortex. The epi-

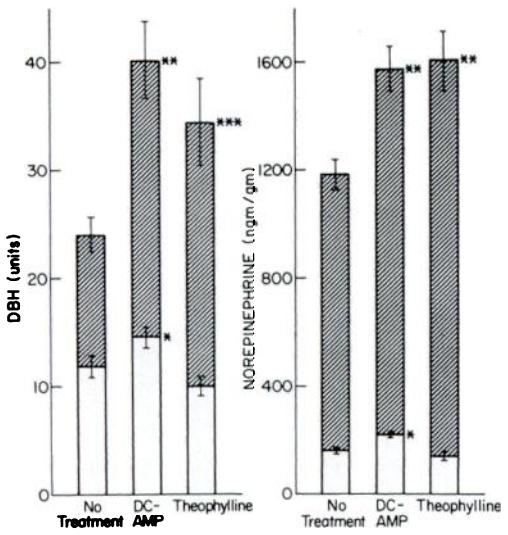

FIG. 2. *Effect of dibutyryl cyclic AMP (DC-AMP) (0.1 mM) and theophylline (1 mM) on dopamine β-hydroxylase (DBH) and norepinephrine release by guinea pig vasa deferentia in incubation medium containing normal calcium concentration*

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 stimulated release of dopamine β-hydroxylase activity and norepinephrine. Each bar represents the mean (± standard error) of at least eight preparations.

* Value exceeds untreated, control preparations ($p < 0.05$).

** Value exceeds untreated, stimulated preparations ($p < 0.01$).

*** Value exceeds untreated, stimulated preparations ($p < 0.05$).

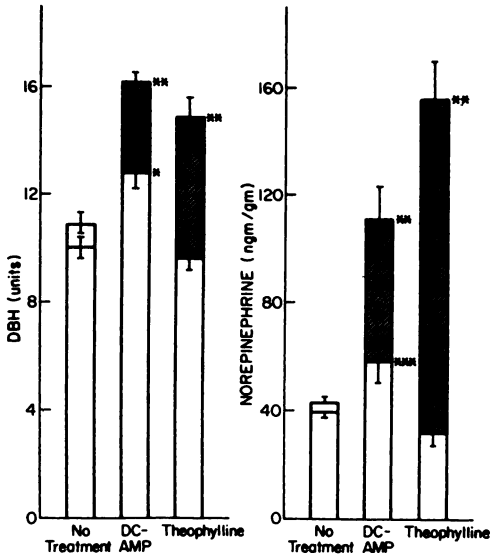

FIG. 3. *Effect of dibutyryl cyclic AMP (DC-AMP) (0.1 mM) and theophylline (1 mM) on dopamine β-hydroxylase (DBH) and norepinephrine release from guinea pig vasa deferentia in calcium-free incubation medium*

The lower, open bars represent the bath concentration for unstimulated organs. Total bars represent total activity recovered after 1 hr of hypogastric nerve stimulation. Shaded areas represent stimulated release of dopamine β-hydroxylase activity and norepinephrine. Each bar represents the mean (± standard error) at least eight preparations.

* Value exceeds untreated, control preparation ($p < 0.001$).

** Value exceeds untreated, stimulated preparations ($p < 0.001$).

*** Value exceeds untreated, control preparations ($p < 0.05$).

nephrine-induced increase in intracellular cyclic AMP in the parotid gland proceeds in the absence of extracellular calcium (3), but extracellular calcium is required for ACTH stimulation of adenyl cyclase in the adrenal cortex (27, 28). Elevation of intracellular cyclic AMP levels in the insect salivary gland does not result in secretion of potassium ion in the absence of extracellular calcium (3, 4), but in the adrenal medulla cyclic AMP and theophylline stimulate the release of catecholamines even in the absence of extracellular calcium (10).

Our findings demonstrate that both dibutyryl cyclic AMP and theophylline enhance stimulation-induced release of dopamine β-hydroxylase from sympathetic nerves in the presence of physiological concentrations of calcium; dibutyryl cyclic AMP, but not theophylline, also enhances spontaneous release of the enzyme. In the absence of extracellular calcium, stimulation-induced release of dopamine β-hydroxylase is completely blocked. Even in the absence of extracellular calcium, however, dibutyryl cyclic AMP increases both spontaneous and stimulation-induced release of dopamine β-hydroxylase. In the calcium-free medium, theophylline elevates only stimulation-induced release of the enzyme.

The failure of exogenously administered

cyclic 3′,5′-AMP to enhance release of dopamine β-hydroxylase while dibutyryl cyclic AMP increased both spontaneous and stimulation-induced release of the enzyme may be related to the greater cell membrane permeability and greater resistance to phosphodiesterase of the dibutyryl derivative (29). At least three different mechanisms may be suggested to explain the effects of dibutyryl cyclic AMP: (a) it may act to release intracellular, bound calcium, which then becomes available to trigger the release mechanism even in the absence of extracellular sources of the ion (10, 17); (b) dibutyryl cyclic AMP may share with cyclic 3′,5′-AMP an ability to release norepinephrine and dopamine β-hydroxylase directly, independent of calcium; (c) dibutyryl cyclic AMP might have direct releasing ability not shared by cyclic-3′,5′-AMP, thus raising the possibility that the dibutyryl derivative does not interact by the same mechanism as cyclic-3′,5′-AMP in the release process at sympathetic nerve endings.

Presumably theophylline, a methylxanthine derivative, increases intracellular 3′,5′-AMP levels by inhibiting phosphodiesterase (6). The resultant increased cyclic AMP levels may then initiate exocytosis by one of the mechanisms described above. Alternatively, theophylline may have a direct effect of mobilizing intracellular calcium (17) or of acting alone to initiate catecholamine release. The failure of theophylline to enhance spontaneous release, as did dibutyryl cyclic AMP, suggests that a stimulus is required to activate adenyl cyclase and increase intracellular cyclic 3′,5′-AMP levels before the effect of phosphodiesterase inhibition is reflected by increased exocytosis.

A general mechanism may thus be proposed for the events that couple excitation and secretion at sympathetic nerve endings. Depolarization of the neuronal membrane by an electrical impulse causes an influx of extracellular calcium and activation of membrane-bound adenyl cyclase, resulting in increased intracellular cyclic 3′,5′-AMP levels. Besides mobilizing intracellular calcium and thus acting in series with calcium, cyclic AMP may act directly in parallel with calcium to activate the presumed neurotubule-neurofilament-vesicle complex (18, 30), resulting in exocytosis, the rapid extrusion of the soluble vesicular contents through the neuronal membrane by a contractile process (18, 30).

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