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SHORT COMMUNICATION

A Role for the Endogenous Activator of 3',5'-Nucleotide Phosphodiesterase in Rat Adrenal Medulla

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SUMMARY

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A procedure for the assay of the activator of cyclic 3',5'-nucleotide phosphodiesterase is described. The activator is stable at 76° for several minutes; this property was used to isolate and measure the activator in rat adrenal medulla. Doses of carbamylcholine or reserpine that increase the adenosine cyclic 3',5'-monophosphate (cAMP) content of adrenal medulla also increase the content of the phosphodiesterase activator. The increase in cAMP content precedes that of the activator. When the increase of the activator reaches peak values, the cAMP content in medulla is declining. It is suggested that in adrenal medullae of rats the content of cAMP can be regulated by a transsynaptic activation of adenylyl cyclase and by an increase in the potency of the cyclic 3',5'-nucleotide phosphodiesterase activator.

Cyclic 3',5'-nucleotide phosphodiesterase is the enzyme system that catalyzes the hydrolysis and inactivation of cyclic nucleotides (1). Conceivably, this enzyme plays an important role in determining the extent and duration of the increase of cyclic nucleotides resulting from the activation of adenylyl cyclase by hormones and neurotransmitters.

The activity of cyclic 3',5'-nucleotide phosphodiesterase is regulated by the amount of an endogenous protein activator, which is present in every tissue along with the enzyme (2-5). The partial inactivation of the phosphodiesterase during purification procedures appears to be due to the dissociation of the activator from the enzyme (2, 6). The addition of the activator protein to purified phsophodiesterase markedly increases the enzyme activity (2, 3, 7, 8). The properties of the endogenous activator of cyclic 3',5'-nucleotide phosphodiesterase and the mechanism whereby it activates this enzyme *in vitro* were studied extensively (2, 3, 6, 9-12). The general consensus is that the activator can change the kinetic properties of the phosphodiesterase. However, there is a lack of information on how the amount of phosphodiesterase activator present in cells is regulated and on the factors involved in its dynamic association with the enzyme.

To elucidate whether the endogenous activator regulates cyclic 3',5'-nucleotide phosphodiesterase *in vivo*, we studied the activity of the protein activator in rat

adrenal medulla at various times after the injection of drugs that increase the adenosine cyclic 3',5'-monophosphate content. We chose adrenal medulla because it contains a uniform cell composition and responds to sustained stimulation of nicotinic receptors with a marked elevation of cAMP.[1] This response has been studied in detail after injections of reserpine or carbamylcholine (13–15).

To isolate the protein activator of cyclic 3',5'-nucleotide phosphodiesterase we took advantage of the thermostability of this activator, which is greater than that of the enzyme (2–4). The adrenal glands of rats were removed after decapitation, and the medulla was separated from the cortex according to Guidotti and Costa (13). In order to preserve the compartmentation of the activator *in vivo* and to prevent its combination with the phosphodiesterase during homogenization, we exposed the medulla to 76° before homogenization. The thermostability of both protein activator and enzyme was studied by incubating the adrenal medulla at 76° for various time periods in test tubes containing 5 ml of 1 mM Tris, pH 7.5, and 1 mM Mg^{2+}. The incubation was terminated by placing the tubes at −80°. The tissue was homogenized in 400 μl of chilled, deionized water and centrifuged at 4° and 12,000 × *g* for 30 min. The supernatant was assayed for both phosphodiesterase activity and activator content. The phosphodiesterase activity was measured by the isotopic method of Filburn and Karn (16). The content of phosphodiesterase activator in the adrenal medulla supernatant was assayed by measuring the stimulation elicited by a standard amount of activator on a purified brain phosphodiesterase. This enzyme preparation was isolated from rat brain according to the procedure of Uzunov and Weiss (7). The incubation mixture used to measure the adrenal medulla content of the endogenous phosphodiesterase activator contained 100 μM cAMP, 5 pmoles of [^{3}H]cAMP (80,000 cpm), 5 mM Mg^{2+}, 10 μl of purified phosphodiesterase containing 1 μg of protein, and 20 μl of adrenal medulla supernatant, in a total volume of 100 μl. The phosphodiesterase activator was measured by comparing the phosphodiesterase activity of the standard mixture containing only the purified enzyme with that of the standard mixture containing in addition various dilutions of the adrenal medulla supernatant. The unit of activity for estimation of the activator was defined as the amount of protein which stimulates by 50% the phosphodiesterase activity of 1 μg of protein of the purified enzyme under the assay conditions described above.

The thermostabilities of the phosphodiesterase activator and of the endogenous phosphodiesterase of rat adrenal medulla were compared (Fig. 1). The enzyme was completely inactivated by 30 sec of incubation at 76°, whereas the activity of the activator protein remained practically unchanged during this incubation. The data presented in Fig. 1 show that an incubation at 76° for longer than 45 sec reduced the phosphodiesterase activator potency by about 40%. No further reduction of the activator potency was obtained by prolonging the incubation. It might be inferred from these data that there are two forms of the phosphodiesterase activator, one more thermostable than the other. Since the activator binds to the phosphodiesterase, we studied whether the enzyme inactivated by incubation at 76° for 30 sec could still bind the activator. We have shown that when the purified phosphodiesterase is denaturated by exposure to 76° it fails to bind to the activator. The procedure used, therefore, allows the separation and assay of phosphodiesterase activator.

The relation between the degree of stimulation of purified phosphodiesterase and the concentration of the activator isolated from rat adrenal medulla is shown in Fig. 2. These data confirm other reports (6, 9, 10) showing that the phosphodiesterase protein activator acts in the presence of Ca^{2+}. The slight activity of the activator in the absence of Ca^{2+} was attributed to the trace of Ca^{2+} present in the reaction mixture. In the presence of 10 μM Ca^{2+} the extent of phosphodiesterase stimulation increased with the amount of activator;

[1] The abbreviation used is: cAMP, adenosine cyclic 3',5'-monophosphate.

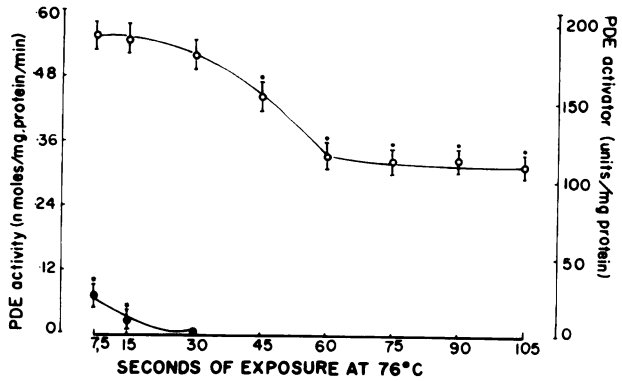

FIG. 1. *Cyclic 3',5'-nucleotide phosphodiesterase (PDE) activity and endogenous phosphodiesterase activator in rat adrenal medulla incubated for various times at 76°*

Each point represents the mean value of eight experiments. Vertical bars indicate standard errors. O——O, activator; ●——●, phosphodiesterase activity.

* $p < 0.05$ when compared with exposure at 37° for 1 min.

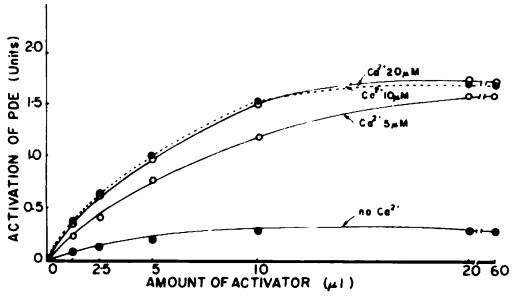

FIG. 2. *Activation of purified phosphodiesterase (PDE) by various amounts of activator isolated from rat adrenal medulla; effect of Ca^{2+}*

The tissue was exposed at 76° for 25 sec, and the activator was isolated and assayed as described in the text. Each point represents the mean value of 10 experiments.

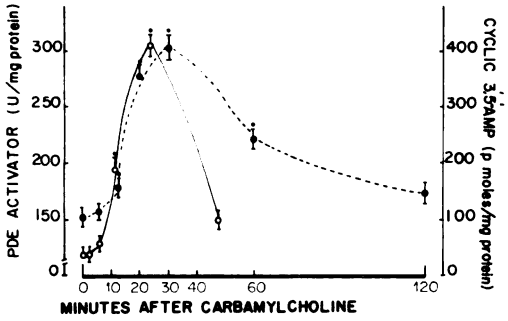

FIG. 3. *Phosphodiesterase (PDE) activator and cAMP content in adrenal medullae of rats treated with carbamylcholine*

Rats were injected with carbamylcholine (5.4 μmoles/kg, intraperitoneally) and killed at various times afterward. Each point represents the mean value of six experiments. The cAMP concentration was measured as described by Guidotti and Costa (13). Vertical bars indicate standard errors. ●- - -●, activator of phosphodiesterase; O——O, cAMP concentration.

* $p < 0.05$ when compared with 0.9% NaCl-treated controls.

saturation was reached with about 20 μl of supernatant (8 μg of protein). When the concentration of Ca^{2+} was increased to 20 μM the potency of the activator was no greater than in presence of 10 μM Ca^{2+}.

The data of Fig. 3 show the relationship between the concentrations of cAMP and the activity of the endogenous activator in adrenal medullae of rats killed at various times after treatment with carbamylcholine (5.6 μmoles/kg, intraperitoneally). It is notable that the increase in potency of the phosphodiesterase activator followed the increase in cAMP content. Moreover, the potency of the activator reached a peak after the cAMP content of medulla began to decline.

The time course of the changes in cAMP content and potency of the phosphodiesterase activator in adrenal medullae of rats killed at various times after treatment with reserpine (Fig. 4) showed a relationship similar to that described in Fig. 3. However, after reserpine treatment the increase

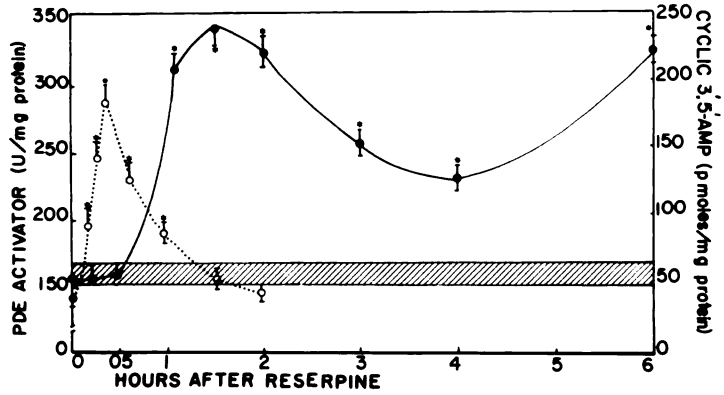

FIG. 4. *Phosphodiesterase (PDE) activator and cAMP content in rat adrenal medulla at various times after reserpine*

Reserpine was administered at a dose of 16 μmoles/kg, intraperitoneally. Each point represents the mean value of eight experiments. Vertical bars indicate standard errors. ●——●, activator of phosphodiesterase; O····O, cAMP concentration.

* $p < 0.05$ when compared with 0.9% NaCl-treated controls.

in potency of activator was delayed in comparison with that in adrenal medullae from animals receiving carbamylcholine. Moreover, after reserpine (Fig. 4) we observed a secondary, delayed peak in the potency of the activator, which occurred 6 hr after drug injection. Probably reserpine has some direct and some rebound effects on the process that brings about the increase in the activator protein. Further studies of the mechanisms regulating the increase in potency of the phosphodiesterase activator are in progress.

In conclusion, we have presented a sensitive assay to measure the endogenous phosphodiesterase activator in rat adrenal medulla. When the cAMP content in rat adrenal medulla is increased by several fold, the potency of the activator also increases. Since the increase of cAMP concentration precedes that of the phosphodiesterase activator (Fig. 3), it may be inferred that when the cAMP content exceeds a certain limit it can regulate its own metabolism by increasing either the activity or the content of the activator. It appears that this activation of phosphodiesterase may have significance in regulating the duration of the increase of cAMP content in medulla. This finding may help to explain why, when rats are exposed to cold at 4°, the duration of the increase in cAMP content of adrenal medulla appears unrelated to the duration of the stimulus (17–19). In support of the view that an increase of cAMP content increases the potency of the phosphodiesterase activator is the preliminary finding that the injection of reserpine but not of carbamylcholine fails to increase the phosphodiesterase activator in denervated medullae of rats. It was reported that after denervation carbamylcholine can still increase the cAMP content of rat adrenal medullae, whereas reserpine fails to change the cAMP content in this tissue (13–15).

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