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COMMENTARY

TRANS-SYNAPTIC REGULATION OF TYROSINE 3-MONO-OXYGENASE BIOSYNTHESIS IN RAT ADRENAL MEDULLA

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In neurons the regulation of transmitter biosynthesis is synchronized with their activity rate; perhaps an increase in the cyclic nucleotide content or changes in specific ion fluxes are operative in coupling the rate of transmitter biosynthesis with neuronal activity [1–3]. In this regulation, adaptive increases in the biosynthesis of enzymes that synthesize neurotransmitters are also important. Often, such increases in protein synthesis are triggered by a persistent acceleration of afferent neuronal activity [1, 3–5]. For instance, when environmental stimuli impose a sustained increase in the secretion rate of the catecholamines stored in adrenal medulla, the synthesis of tyrosine-3-mono-oxygenase or tyrosine hydroxylase (TH) which is believed to be a major factor in maintaining catecholamines at steady state [6] is increased [4]. Since the increase in TH synthesis in chromaffin post-synaptic cells of medulla is elicited by an increase in the activity of afferent neurons, this process has been termed trans-synaptic induction of TH [4]. In adrenal medulla, the uniformity in the afferent innervation of chromaffin cells and the limited number of cell types facilitate the search for the biochemical events which couple rates of chromaffin cell stimulation with the biosynthesis rate of TH. As a long-range objective in the studies of TH regulation in medulla, we have to elucidate how synaptic stimuli regulate gene expression coding for TH. As a corollary of this commentary, we are suggesting a model which depicts how, in chromaffin cells, the rate of nicotinic receptor stimulation modifies the mRNA synthesis in the nucleus. In this model we have also attempted to single out the temporal and spatial sequence of intracellular molecular events whereby the biosynthesis of new mRNA, including that which regulates TH biosynthesis, is promoted by trans-synaptic stimuli. The model also proposes that the first (acetylcholine) and second (3′,5′-cyclic adenosine monophosphate, cAMP) messengers are involved in the trans-synaptic induction of TH and stresses the role of protein kinases in the regulation of poly A RNA synthesis which precedes the increase in TH synthesis. Although it may be reasonable to assume that mechanisms similar to those of chromaffin cells may be operative also in adrenergic neurons of the central nervous system (CNS), a verification of this similarity has not been obtained.

Genetic mechanisms in the trans-synaptic induction of TH

It was Julius Axelrod *et al.* [4, 7, 8] who discovered that, in the adrenal medulla, the persistent neurally mediated release of acetylcholine onto post-synaptic nicotinic receptors was the signal for the induction of TH. Since a blockade of RNA translation or transcription, at appropriate times, inhibits the increase of medullary TH elicited trans-synaptically, it was proposed that in adrenal medulla an increase in the number of TH molecules was responsible for the induction of TH [4, 9, 10]. This proposal was upheld by showing that in adrenal medulla during TH induction there was an increase in the number of molecules that were precipitated by an antibody directed toward TH [11, 12]. Successive studies have shown that, during the trans-synaptic induction of TH, the synthesis rate of the TH molecules was increased while their rate of degradation remained unaltered [13]. Since the synthesis rate of TH was increased over a period of about 9–30 hr after application of an inducing stimulus, we studied the possibility of poly A RNA being increased after the application of the inducing stimulus. We found that in rats the rate of poly A RNA biosynthesis dependent on RNA polymerase II was increased already at 6 hr, reached a maximum at 8–10 hr and was terminated at 16 hr after exposure to 4° [14]. This increase was abolished ipsilaterally to the transection of the afferent nerves to the adrenal medulla [14]. These findings are consistent with the hypothesis that nuclear chromatin is the site where an important step for the trans-synaptic regulation of TH synthesis takes place and that cold exposure, by increasing the release rate of acetylcholine, activates nicotinic receptors and thereby brings about an increase in the formation of messenger RNA coding for TH synthesis.

Participation of cyclic AMP (cAMP) and cyclic GMP (cGMP) in the trans-synaptic induction of medullary TH

Since a gene regulation hypothesis [4, 5] had been proposed to explain the trans-synaptic induction of medullary TH, we began to search for a suitable mechanism whereby synaptic activity could be transduced into a signal that changes the synthesis rates

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of nuclear RNA coding for TH and for other specific proteins of chromaffin cells.

Using as a stimulus cold exposure or the injection of carbamylcholine, or reserpine with and without a pretreatment with ganglionic blocking agents, it was established that various stimuli became operative in inducing TH if they persisted for about 90 min [15, 16]. We reasoned that the biochemical signal involved in transducing the membrane depolarization into a change in mRNA synthesis should last for about 90 min. Moreover, while the onset of the biochemical response should be coupled with the stimulus, its duration should be about 90 min and it should be otherwise independent of the duration of the inducing stimulus. The biochemical signal occurring in adrenal medulla in conjunction with trans-synaptic induction of TH which closely fulfills the theoretical requirement defined above was an increase of medullary cAMP content [3, 15–17].

A stimulation of nicotinic receptors by carbamylcholine or by drugs which release acetylcholine from splanchnic nerve terminals elicits a sharp increase in the cAMP content of rat adrenal medulla (see Fig. 2B and Table 1) [16–18]. While the onset of the increase in medullary cAMP content is coupled with the stimulus, its duration is independent of the time course of the stimulus. The cAMP increase never exceeds 90 min even though the stimulus lasts for several hours [15–20]. All the stimuli that induce TH increase medullary cAMP content, but they change the cGMP content in both directions; moreover, the direction of the cGMP change is unrelated to the TH induction and its intensity is modest when compared to that of the increase in cAMP [16, 19, 20]. Using specific receptor blockers, we have established that, in medulla, the stimulation of nicotinic receptors causes accumulation of cAMP, whereas the stimulation of muscarinic receptors causes the accumulation of cGMP [18–20]. Using specific stimulants of the two receptors, the cAMP/cGMP ratio increases or decreases after the stimulation of nicotinic or muscarinic receptors respectively. Since the cAMP/cGMP ratio increases after stimuli that induce TH, the characteristics of the second messenger response uphold earlier proposals [4] that the stimulation of nicotinic receptors mediates TH induction.

In adrenal medulla, the duration of the cAMP/cGMP increase appears to be regulated by the release from binding sites in synaptic membranes of an endogenous protein. This protein in the presence of 20 μM Ca^{2+} increases the affinity of the high K_m cyclic nucleotide phosphodiesterase for cAMP. Since this high K_m enzyme has a high V_{max}, the metabolism *in vivo* of cAMP is accelerated following the release of this endogenous protein from binding sites in the membrane [21–23].

The fundamental role of cAMP or the cAMP/cGMP concentration ratio in the mechanisms leading to the induction of medullary TH is suggested by the following findings: (1) all the stimuli that induce TH increase the cAMP/cGMP ratio of medulla for a threshold duration of 1–2 hr (see Table 1) [20, 21, 24]. Injections of propranolol [25], dopamine [26], ACTH [26, 27], or of small doses of carbamylcholine [28], which fail to induce TH, increase the

Table 1. Relationship between the increase in cAMP content, the increase in cytosol protein kinase catalytic subunits, the increase in nuclear protein kinase activity and the tyrosine-3-mono-oxygenase induction in rat adrenal medulla*

		% cAMP increase			PK catalytic subunits in cytosol		Increase of nuclear PK activity	TH
Stimulus	Denervation	30 min	60 min	240 min	At 2 hr	At 6 hr	Time of maximal effect	At 24 hr
Exposure to 4°	No	470	120	0	↑	—	8	↑
	Yes	50	0	0	—	—	—	—
Repeated swimming stress	No	0	0	700	—	↑	12	↑*
Reserpine (8 μmoles/kg i.p.)	No	180	70	0	↑	—	8	↑
	Yes	25	0	0	—	—	—	—
Reserpine (16 μmoles/kg i.p.)	No	310	100	0	↑	—	8	↑
Propranolol (40 μmoles/kg, i.p.) + reserpine (16 μmoles/kg, i.p.)	No	425	250	0	↑	—	8	↑
Dexamethasone (1 μmoles/kg, i.p.) + reserpine (16 μmoles/kg, i.p.)	No	100	0	120	—	↑	16	↑†
Carbamycholine (3 μmoles/kg, i.p.)	No	900	0	0	—	—	—	—
Carbamylcholine (9 μmoles/kg, i.p.)	No	1300	300	0	↑	—	8	↑
	Yes	1200	250	0	↑	—	8	↑
Carbamylcholine (9 μmoles/kg, i.p.) + atropine (4 μmoles/kg, i.p.)	No	1000	150	0	↑	—	8	↑
Hexamethonium (4 μmoles/kg, i.p.) + carbamylcholine (9 μmoles/kg, i.p.)	No	100	0	0	—	—	—	—
Aminophylline (200 μmoles/kg, i.p.)	No	1000	600	—	↑	—	8	↑
	Yes	470	250	—	↑	—	—	—
Dopamine (50 μmoles/kg, s.c.)	No	125	0	0	—	—	—	—
ACTH (1 I.U./kg, i.v.)	No	115	0	0	—	—	—	—
Propranolol (40 μmoles/kg, i.p.)	No	700	0	0	—	—	—	—

* The symbols are defined as follows: (↑) increase in number of enzyme molecules; and (—) response not present.
† Maximum increase occurs after 32 hr.

cAMP/cGMP ratio of medulla by an extent comparable to that elicited by a dose of reserpine which induces TH. However, these stimuli elicit a second messenger response lasting less than 30 min (Table 1), whereas reserpine causes a longer lasting increase of medullary cAMP content; (2) high doses of corticosteroids delay the increase of medullary cAMP/cGMP ratio and the onset of the increase in TH activity elicited by reserpine [29]; (3) blockade of nicotinic receptors or denervation inhibits the increase of medullary cAMP/cGMP ratio and the induction of TH [15, 16, 20, 30]; and (4) carbamylcholine increases the cAMP content and induces TH in adrenal medullae which were denervated 5 days before the experiment [18, 31]; however, this drug fails to elicit either response if the denervation was performed 15 days before the experiment [32]. This parallelism could be expected if adenylate cyclase and nicotinic receptors were the components of the same supramolecular complex. This view is in line with reports that medullary adenylate cyclase in a cell free system is not activated by acetylcholine [31, 33, 34], but the enzyme is activated by acetylcholine added to medullary slices [26, 31].

The findings discussed above suggest that it could be highly misleading to evaluate the involvement of cAMP in the trans-synaptic induction of TH by measuring the cAMP/cGMP ratio in medulla at a fixed time interval after the inducing stimulus. The necessity of providing a complete time course of the second messenger response and of the increase in TH activity was documented by experiments with swimming stress [35, 36] or with injections of corticosteroids and reserpine [29, 37] (see also Table 1).

It has been suggested that the increase in cAMP content measured in medulla after cold exposure is not elicited by the stimulation of nicotinic receptors, but reflects a diffusion of cAMP from cortical tissue to medulla [26]. However, denervation virtually abolishes the increase in cAMP content elicited in medulla by reserpine and cold exposure but not the increase of cAMP content elicited by these stimuli in adrenal cortex [18, 20, 30, 31]. It is conceivable that the mechanism that regulates the increase in medullary cAMP content is independent of that which causes the increases of cAMP in adrenal cortex. Therefore, the "trans-synaptic theory" of the medullary increase of cAMP content [15–20] appears to be more consonant with experimental results than the "diffusion theory" [36, 37].

Significance of cAMP dependent protein kinase (PK) activation in the trans-synaptic induction of TH

The time sequence of the biochemical events which have been measured in the adrenal medulla in the time interval between the stimulus application and the induction of TH (Fig. 1) shows that there is an interval of several hours between the termination of the increase in the cAMP/cGMP concentration ratio and the increase in the poly A RNA synthesis. In fact, in no instance could we find that the change in the cAMP/cGMP concentration ratio lasted longer than 2 hr, and in no instance did the increase in

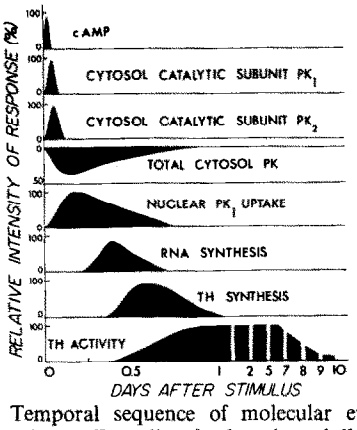

Fig. 1. Temporal sequence of molecular events taking place in chromaffin cells of adrenal medulla during the trans-synaptic induction of tyrosine hydroxylase (TH). After application of a stimulus (exposure to 4°; reserpine, carbamylcholine), cAMP increases for about 90 min. The cAMP-dependent protein kinases, type 1 (PK_1) and type 2 (PK_2), of cytosol are activated and release their catalytic subunits for 2–3 hr. This activation is followed by a decrease of the total cytosol PK activity and by a nuclear uptake of the catalytic subunit of PK_1. The increase of nuclear PK is initiated before the increase in RNA synthesis, lasts for about 18 hr and precedes the increase in TH synthesis and TH activity.

mRNA synthesis occur prior to 5–6 hr from the stimulus application [14]. This suggests that the stimulus-coupled increase in the cAMP content of medulla triggers one or more intermediate metabolic responses in chromaffin cells which, in turn, promote new synthesis of RNA. To broaden the present understanding of how cAMP regulates communications between events occurring in chromaffin cell membrane and in the nuclei, we have studied in detail the dynamics of the PK activation during the time period elapsing from the increase in cAMP content elicited trans-synaptically to the beginning of the increase in RNA synthesis. Since it is currently believed that in eukariotic cells the regulatory subunits of PKases are a generalized receptor for cAMP [38–41], we have explored whether, *in vivo*, following the increase in medullary cAMP elicited trans-synaptically there is an activation of cAMP-dependent PK concomitant with a dissociation of free catalytic subunits of PK.

Using gel filtration and ion exchange chromatography [41–43], it is possible to isolate the cAMP-dependent PK present in tissue homogenates and to distinguish whether the kinase activity is due to catalytic subunits of cAMP-dependent PKases or to kinase molecules which are cAMP independent. By these techniques we have determined that about 75 per cent of the PK molecules present in the cytosol of rat adrenal medulla are cAMP dependent [25, 28]. When the cytosol of adrenal medulla from control rats was chromatographed with DE-52 cellulose ion exchange resin and eluted with an NaCl gradient, two peaks of cAMP-dependent PK activity were distinguished. The first peak was eluted by 0.1 M NaCl and the second by approximately 0.2 M NaCl suggesting that they correspond to PK_1 and PK_2 according to the nomenclature of Corbin *et al.* [43]. The PK_1 represented about 40 per cent and the PK_2 about 60 per cent of the total cytosol activity;* only

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20 per cent of the cAMP-dependent PK_1 or PK_2 molecules were present in the activated state as free catalytic subunits, while 80 per cent of the molecules were in the inactive state [28, 44].

An inactive cAMP-dependent PK is a high molecular weight form of PK, composed of regulatory and catalytic subunits held together by ionic bonds [45]. All the trans-synaptic signals that elicit an increase of cAMP content and determine an induction of TH 24 hr later also produce an early activation of the cytosol cAMP-dependent kinases with dissociation of free regulatory and catalytic subunits of PK_1 and PK_2 (Figs. 1, 2B, and Table 1; see also Refs. 28, 44 and 46). By studying stimuli of increasing intensity, and measuring PK at various times, we have found that the number of free catalytic subunits present at 2 hr correlates with the degree of the TH induction 24 hr later [25, 28, 44, 46]; moreover, the increase in the number of free catalytic subunits of PK in cytosol persists several hours longer than the increase in cAMP content (Figs. 1 and 2; see also Refs. 25, 28 and 44).

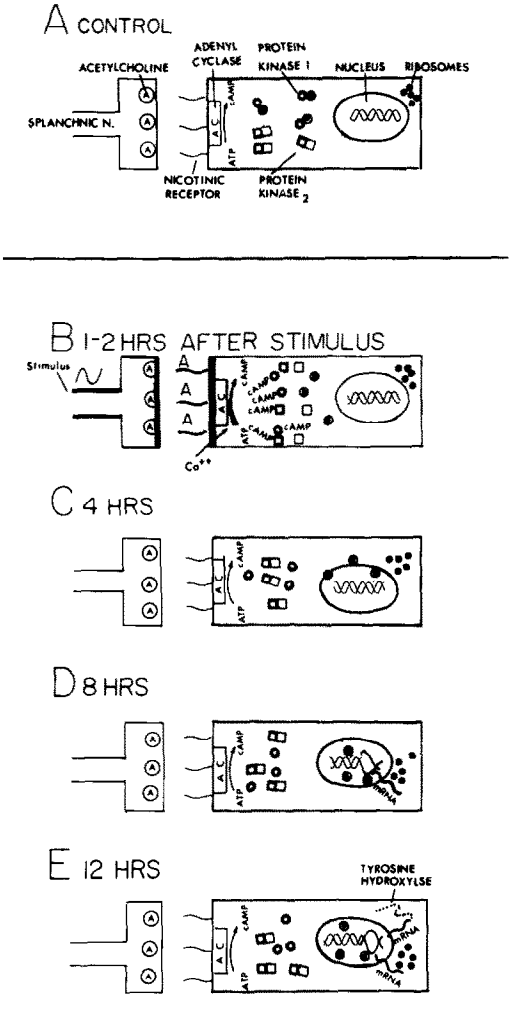

Fig. 2. Response of chromaffin cells of adrenal medulla to trans-synaptic stimuli. Control (A). The acetylcholine released from the splanchnic nerve terminals interacts with nicotinic receptors located on the membrane of chromaffin cells (B). The stimulation of nicotinic receptors results in an increase of cAMP content and in the activation of cytosol protein kinase 1 and 2 which dissociate into regulatory (□, •) and catalytic subunits (□, ○) (B). After 2 hr the increase of cAMP is extinguished and at 4 hr the cytosol protein kinase 2 is reassociated (C). However, the catalytic subunits of protein kinase 1 migrate from cytosol (C) and are taken up by the nucleus (D). The uptake of protein kinase 1 catalytic subunits by the nucleus causes the phosphorylation of chromosomal proteins and increases mRNA synthesis (D). The increase of mRNA synthesis precedes the synthesis of new tyrosine hydroxylase molecules (E).

In adrenal medulla, the cAMP content ranges from 1.5 to 2 μmoles/kg wet weight. Stimuli which induce TH increase cAMP content to about 5–10 μmoles/kg wet weight [18]. In medullary homogenates, the concentration of cAMP which activates PK half maximally, is in the range of 0.20–0.3 μM [25]. However, we observed that when the adrenal medullae from normal rats were homogenized in 3 vol. of isotonic sucrose, although the concentration of cAMP may be greater than 0.3 μM, only 20 per cent of the protein kinase was present as a free catalytic subunit [25]. As proposed by Beavo *et al.* [47, 48], the PK activation does not depend exclusively on the concentration of cAMP but also depends on the tissue concentrations of PK and on an endogenous heat-stable small molecular weight protein which inhibits PK activity [49]. From the data published by Beavo *et al.* [47, 48] and Walsh and Ashby [49], one might predict that, if the concentrations of endogenous inhibitor were sufficient to inhibit 20 per cent of the catalytic subunits of PK molecules present, only an activation involving more than 20 per cent of the inactive cAMP-dependent enzyme molecules can be expressed. Obviously, the tissue content of this endogenous PK inhibitor together with the cAMP concentration regulates the expression of the second messenger response. Conceivably, a decrease in endogenous inhibitor facilitates PK activation in the presence of a small increase of cAMP content, and an increase of the amount of inhibitor limits the amount of protein phosphorylation that can be expressed by a given increase in medullary cAMP content. An appreciation of these possibilities convinced us that this could be an additional cuase of error when the measure of cAMP content is taken as an index of the involvement of cAMP in the mediation of the trans-synaptic induction of TH.

To avoid being misled by measuring cAMP, one can assess whether at various times after the stimulus there is an activation of the cAMP-dependent protein kinase in cytosol. A demonstration that many drugs can increase the medullary cAMP content without increasing the number of free catalytic subunits of PK is shown in Table 1. Whenever the cAMP increase lasted less than 30 min, it was not followed by an increase in the number of the free catalytic subunits of PK and by an induction of TH; however, whenever the increase of cAMP lasted 60 min or longer, the PK was activated for about 2 hr and TH was induced. These results suggest that PK activation is the intermediary process preceding the increase in RNA synthesis elicited trans-synaptically. Moreover, it appears that both duration and intensity of cAMP increase regulate the induction of TH through an activation of PK.

Nuclear uptake and retention of cAMP-dependent catalytic subunits, an essential process for the trans-synaptic induction of medullary TH

A variety of molecular forms of PKases have been found in nuclei of eukariotic cells (for a review, see Ref. 50). Most of these kinases phosphorylate nuclear proteins in a cyclic nucleotide independent fashion. Also, since in rat adrenal medulla the nuclear phosphorylation is not regulated by cAMP* [46, 51, 52] the possibility that an increase of cAMP may influence nuclear phosphorylation and, consequently regulate RNA transcription, may appear paradoxical. The apparent paradox can be reconciled by the observation that specific nuclear protein phosphorylation may be achieved as a consequence of a redistribution in the cytosol of cAMP-dependent PKases. Based on these assumptions, the PK activation could be viewed as a third messenger in the trans-synaptic induction of TH. In fact, this activation is an essential prerequisite for the nuclear uptake of free catalytic subunits of PK_1, which precedes the new synthesis of nuclear RNA.

After the stimulus-induced rise of medullary cAMP and the subsequent dissociation of PK_1 and PK_2, we observed that the total cytosol PK activity measured in the presence of cAMP fell* [28, 46, 53] (Fig. 1). This decrease was already significant 2 hr after the stimulus application when the activation of PK_1 and PK_2 was maximal, reached a peak at about 4 hr when the regulatory and catalytic subunits of PK_1 and PK_2 were reassociated, and persisted longer than 12–18 hr [28, 44, 46, 53] (Fig. 1). At 7 hr after various stimuli that induce TH, 30–40 per cent of the PK activity disappeared from the cytosol [28, 44, 46]. When the cytosol of adrenal medullae from rats killed 7 hr after an inducing stimulus was applied to a DE-52 cellulose ion exchange column, we found that the PK_1 virtually disappeared while the PK_2 was unchanged.* It is important to stress that the number of free catalytic subunits in medullary cytosol had returned to normal several hours earlier [28].

As reported in Figs. 1 and 2C, D and E, when the cytosol PK_1 was decreased, the nuclear PK activity was increased. Nuclei, isolated from medulla of rats injected with reserpine or exposed to cold 7–10 hr earlier, phosphorylated endogenous nuclear proteins at a rate twice as fast as that of nuclei from rats injected with saline.* This increase was maintained when the nuclei were treated to remove contaminant PK, adsorbed from cytosol.*

The medullary nuclei were also extracted with Triton X-100 and 0.5 M NaCl and the PK activity of these extracts was measured [28, 46, 53]. The phosphorylating activity of nuclear extracts prepared from adrenal medullae of rats killed 7 hr after a stimulus that induces TH was greater than that of extracts prepared from adrenal medullae of rats injected with only saline [46]. This difference was nullified by the addition of regulatory subunits of PK and was reinstated by the addition of cAMP to the reaction medium. Moreover, in nuclei purified from rat adrenal medulla, the phosphorylation of endogenous substrates was increased only if they were incubated with PK_1 but not with PK_2. Since, *in vivo*, PK_1 disappeared from cytosol after stimuli that induce TH, and, *in vitro*, only PK_1 can increase nuclear phosphorylation, we have inferred that the increase in phosphorylating activity found in nuclei derives from the nuclear uptake of catalytic subunits of PK_1 (see Figs. 1 and 2D and E).

Recent reports by Palmer *et al.* [54, 55] have shown that perfusion of the liver with glucagon or dibutyryl cAMP elicits a redistribution of protein kinase catalytic subunits: they decrease in cytosol and accumulate in the nuclear fraction. A similar redistribution of PK has been described in calf ovary in response to injections of chorionic gonadotrophines [56]. The proposal was made that the transfer to the nucleus of cytosolic PK can influence nuclear events such as RNA transcription. Usually these studies have dealt with short-term translocation of PK and have never followed in time the evolution of this translocation. Actually, the physiological significance of the rapid shift of PK distribution in these tissues has been questioned by Keely *et al.* [57]. These authors, working with isolated heart preparations, have suggested that the rapidly occurring and the short-lasting translocation of cytosol PK to particulate fractions may be due to artifactual binding of dissociated catalytic subunits of PK; this binding occurs in conjunction with the increase of cAMP and requires the presence of a low salt buffer.

The PK redistribution that we have described in the adrenal medulla was observed when the homogenization was performed in 0.5 M NaCl, lasted for several hours, and persisted after the cAMP increase was back to normal. The nucleus intervenes probably with an active uptake mechanism transferring inside the nuclear membranes a specific molecular form of cytosol PK; we suggest PK_1. Although the exact mechanism involved in this uptake is not readily understood, we have found that the molecular properties of cytosol PK_1 change during the nuclear uptake.* Moreover, an active control is presumably operative in causing the nuclear retention of PK_1 for 12–18 hr. For this reason and to avoid confusion, we have dismissed the term "translocation" to indicate the increase in nuclear enzyme activity in adrenal medulla and prefer the term "nuclear uptake" to indicate, on one hand, the crucial physiological value of this event, and on the other hand, the long-lasting characteristic of this change.

In view of the above, it is now pertinent to ask whether the nuclear uptake and retention of the catalytic subunits of PK_1 could have any physiological significance for the nuclear control of the TH synthesis. The evidence available [53] suggests that in rat adrenal medulla a nuclear directed uptake of a specific catalytic subunit of the cytosol PK is a molecular signal designed to expand and amplify the message brought to a cell membrane by activation of nicotinic post-synaptic receptors: (1) there is a striking correlation between a large variety of experimental conditions that induce TH with a trans-synaptic mechanism and the increase of nuclear phosphorylation (see Table 1); (2) when the cAMP-dependent PK_1 was incubated with nuclei isolated from adrenal medulla, there was an increase in nuclear phosphorylation* [52], and poly A RNA synthesis [52]; (3) the

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increase in nuclear PK activity of medulla reached a maximum at 7 hr after the stimulus application when the synthesis of poly A RNA dependent on RNA polymerase II was increased (Fig. 2); (4) carbamylcholine and aminophylline, by activating nicotinic receptors or inhibiting cyclic nucleotide phosphodiesterase, respectively, increased cAMP content, and activated and translocated cytosol PK_1 (see Table 1). In medulla, denervated 5 days before the experiment, all four responses elicited by carbamylcholine were unchanged. In contrast, aminophylline [53] increased the cAMP content and maximally activated the cytosol PK of denervated medulla but failed to cause a nuclear uptake of PK and to induce TH; (5) experiments performed in the pineal also give support to the specificity of the nuclear uptake of PK_1 catalytic subunits to promote an increase of protein synthesis. When the cAMP content of pineal was increased by isoproterenol and the cytosol PK in this tissue was activated, we failed to observe nuclear uptake of PK and an increase in the protein synthesis. We have studied the properties of pineal protein kinase and found that this tissue virtually does not contain PK_1.* These experiments confirmed the concept that only the catalytic subunits of PK_1 are taken up by the nucleus and promote activation of nuclear RNA synthesis; and (6) *in vivo*, the nuclear retention of PK_1 appears to require innervation. In fact, when the adrenal medulla was denervated during PK translocation, the catalytic subunits left the nucleus promptly [53] and as a result of the decrease in nuclear phosphorylation caused by denervation, TH failed to be induced [53].

Relationship between the increase of cAMP, the activation of PK and the nuclear uptake of free catalytic subunits of PK_1 in the trans-synaptic control of the genetic code expression in rat adrenal medulla

The cascade of temporal and spatial biochemical events leading to TH induction triggered by the activation of nicotinic receptors located on chromaffin cell membranes is schematically depicted in Figs. 1 and 2. After the stimulation of nicotinic receptors, there is an increase in membrane bound adenylate cyclase activity leading to an intracellular increase of cAMP (Figs. 1 and 2B). This newly synthesized cAMP is bound to receptor proteins present in the cytosol which are the regulatory subunits of the two different types (PK_1 and PK_2) of cAMP-dependent PK (Fig. 2B). As a result of this interaction, the inactive undissociated PK_1 and PK_2 release the free active catalytic subunits (Figs. 1 and 2B). The active catalytic subunits phosphorylate the membranes and release in the cytosol the membrane bound protein activator of phosphodiesterase which lowers the K_m of the high K_m enzyme and increases the rate of cAMP degradation. Thus, by a self-regulatory mechanism the second messenger concentratioon returns to basal levels. When the cAMP content is back to normal, the free catalytic subunits of PK in cytosol are still increasing (Fig. 1). Their normalization occurs by two different processes: first, the catalytic subunits of PK_2 reassociate with the regulatory subunits (Fig. 2C), and second, the catalytic subunits of PK_1 are taken up by the nucleus, while the respective regulatory subunits remain in the cytosol (Fig. 2C).

The detailed mechanism whereby catalytic subunits of PK_1 are taken up and retained by the nucleus is unknown. Presumably this mechanism requires some enzymatic process and change in the properties of the kinase. It is still unknown how compounds cross the nuclear membrane, but it is suspected that specific enzymatic mechanisms are operative [58]. We know that the nuclear uptake of PK_1 catalytic subunits precedes the increase in the RNA synthesis and the induction of TH (Figs. 1 and 2C, D and E). The increase of protein kinase subunits in nucleus is terminated after the termination of the increase in mRNA synthesis. We do not have as yet any information as to whether the catalytic subunits taken up by the nucleus are transferred back to the cytosol or if new protein synthesis replaced the cytosol catalytic subunits. The mechanisms regulating the nuclear uptake and retention of the cytosol protein kinase in relation to the regulation of genetic mechanisms coding for TH are now a direct object of our studies.

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