DELAYED INCREASE OF TYROSINE HYDROXYLASE ACTIVITY INDUCED BY TRANSSYNAPTIC

1. INTRODUCTION

The rate of impulse flow in afferent nerves influences metabolic rates of secretory cells. In adrenal medulla chromaffin cells store epinephrine or norepinephrine and secrete these amines into the bloodstream. The rate of this secretion is regulated by cholinergic nerves impinging upon nicotinic receptors located on the chromaffin cell membrane This rate increases during various forms of physical exercise and in certain emotional states. It appears that the increase of adrenal medullary secretion is an index of the intensity but not of the quality of emotion.

STIMULATION IN CHROMAFFIN CELLS: ROLE OF CYCLIC NUCLEOTIDES AS SECOND MESSENGERS

毛志江

In this presentation we have tried to elucidate the molecular nature of the mechanisms whereby adrenal chromaffin cells adjust the synthesis of cate cholamines in order to support a high rate of catecholamine secretion during sustained and intensely charged emotional states. We became interested in this regulation because it could provide a model to study long term enzyme regulation mediated transsynaptically. Such studies could bring about a better understanding of the biochemical mechanisms involved in the trophic action exerted by nerves and give some insight on the biochemistry of long term memory.

II. Control of Tyrosine Hydroxylase Activity

A. Rapid Modulation

It is currently believed that in adrenergic cells, the rate of catecholamine synthesis undergoes rapid changes by resorting to a feedback control by product inhibition Tyrosine hydroxylation, the rate limiting step for catecholamine synthesis, could be modulated by catecholamines which can inhibit tyrosine hydroxylase (EC 1.14.3.) by competing with the required pteridin cofactor However, various considerations have suggested that this feedback control may not be the only operative factor in the rapid control of catecholamine synthesis.

More recently rapid changes in the ratios of the amount of tyrosine hydroxylase free in the cytoplasm and bound to synaptic vesicle membrane has been envisaged as another regulatory mechanism. According to Kuczenski and Mandell and Kuczenski the membrane bound form of tyrosine hydro xylase, compared to the soluble form, exhibits: (a) a smaller apparent k_m for the synthetie cofactor, 2-amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetrapteridine ($DMPH_4$) ; (b) a lower k_i for dopamine,and (c) a lower k_m for tyrosine. It was suggested that the membrane bound enzyme represents a functional form of the enzyme at synapses When heparin is allowed to interact with the soluble tyrosine hydroxylase, it lowers the k_m for $DMPH_i$, decreases the ki for dopamine and increases the apparent Vmax of the enzyme. Moreover, soluble tyrosine hydroxylase can undergo a dramatic conformational transition over a relatively narrow range of hydrogen ion concentration Despite their different characteristics, it appears that the soluble and membrane bound enzymes are the same molecular form of the enzyme. It is not yet known in vivo how the transformation of these two forms of the enzyme regulate catecholamine synthesis in the dopaminergic nerve terminals. In chromaffin cells,the presence of bound tyrosine hydroxylase has been challenged by recent evidence showing that tyrosine hydroxylase only ex!sts in soluble form. Thus whether transformation of the enzyme would occur and play a role in regulating catecholamine synthesis in adrenal remains open for further. study. Weiner et al.have recently reported an immediately increased conversion rate of 3H-L-tyrosine to norepinephrine after extensive nerve stimulation of the isolated and perfused guinea pig vas deferens. These authors believe that this increase is not elicited by a decrease of feedback inhibition involving mechanisms which are competitive with the pteridine cofactor or by physical transformation of tyrosine hydroxylase. Neural st!mulation in this tissue may either reduce interact ion of catecholamines on an allosteric site of the enzyme, or trigger other effector-allosteric site interactions to bring about an increase in tyrosine hydroxylase activity

B. Long term enzyme adaptation

A prolonged increase in the activity of cholinergic nerves afferent to adrenal chromaffin cells brings into play a change in the enzymatic activity of constitutional maoromolecules in these cells. A similar finding was reported in sympathetic ganglion where it is not associated with an increase of the total protein content The change of the macromolecular const itution of the chromaffin cells brought about by persistant cholinergic stimulation can be revealed by an increased activi of tyrosne hydroxyliase and dopamine β-hydroxylase (EC. 1.14.2.1.) measured in

vitro The evidence available thus supports the view that this increase of enz ymatic activities is due to a specific increase of protein synthesis. It is not yet known whether this increase is mediated by a greater than normal formation of specific messenger RNA molecules or by a facilitation of either the initiation step or the rate of polysomal translation.

Usually various conditions that increase tyrosine hydroxylase activity increase also the activity of dopamine-β-hydroxylase Both enzymic activities are dependent upon innervation and pituitary ACTH. Therefore, it may be pertinent to consider whether the genetic information for catecholamine synthesizing enzymes is located in a single operon. Exposure of the rats to 4°C for 1 to 4 days gradually increases both tyrosine hydroxylase and dopamine-β-hydroxylase activivity in adrenal medulla; however, the DOPA decar-boxylase (EC. 4.1.2.26), another enzyme involved in catecholamine biosynthesis fails to increase in parallel. This information suggests that more than one operon may be involved in the genetic code for the enzymes that synthesize catecholamines. Whether the biosynthesis of tyrosine hydroxylase and that of dopamine-β-hydroxylase are regulated by identical or similar mechanisms can not be determined at this moment.

III. The Long Term Increase of Tyrosine Hydroxylase Activity in Adrenal Medulla Induced by Cold Stress

A. Stimulus Duration

Thoenen reported that the medullary tyrosine hydroxylase activity of rats kept at 4°C for a protracted (24-28hrs) time period is greater than that of rats kept at 20°C. We found that exposition of rats to 4°C for only a limited time period was sufficient to cause a delayed long term increase of tyrosine hydroxylase activity in adrenal medulla The data reported in Table 1 and Figure 1 show that exposure to 4°C for one hour is sufficient to increase the tyrosine hydroxylase activity 24 hours later. Since the enzymic activity was not increased in the contro-lateral denervated adrenal (Table 1), we infer that when impulse flow in afferent cholinergic nerves was increased foe one hour, the long term adaptation of medullary tyrosine hydroxylase would ensue. A similar obserrvation was reported also for the long term adaptation of the tyrosine hydroxylase activity of sympathetic ganglia in demedullated adult rats rats Thoenen et al. have confirmed this time constant for stimulus duration using young rats (100 g body weight) and repeated swimming stresses as a stimulus.

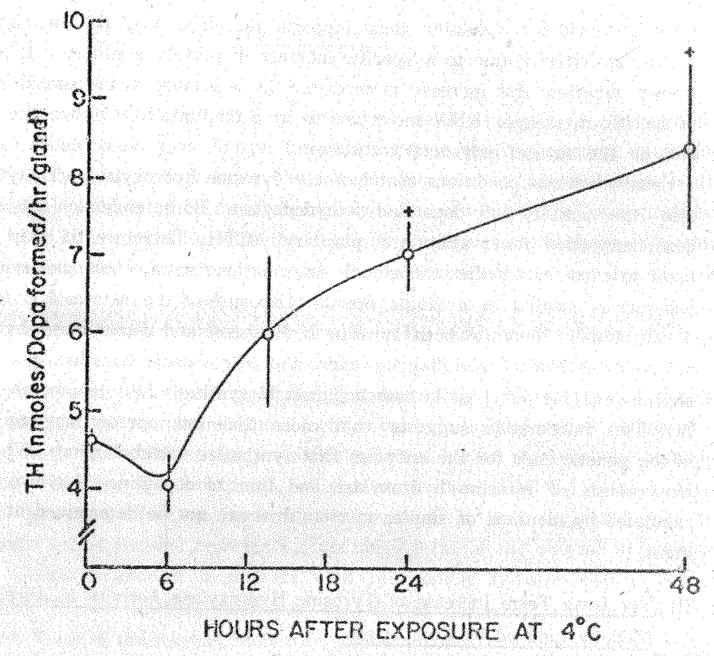

FIGURE 1 Activity of tyrosine hydroxylase in homogenates of adrenal medulla of rats exposed to 4℃ for 1 hour and then killed at various times after this exposure to cold. The rat's fur is wetted with water at 20℃ immediately before exposure at 4℃. Tyrosine hydroxylase activity was assayed in adrenal homogenates, dialyzed for 3 hours at 4℃ accrding to Waymire, Bjur, and Weiner +P<0.05 when compared with control values.

B. Cyclie Nucleotides as Second Messengers for Enzyme Synthesis

In view of the very well known involvement of adenosine-3' ,5'-monophosphate (cAMP) and guanosine-3' ,5'-monophosphate (cGMP) in various types of synaptic transmission, we studied the effects of cold exposure on the concentrations of these nucleotides in the adrenal gland. We have previously reported that the medullary concentrations of cAMP are increased as a result of 4°C exposure of rats. More recently we have reported that cold exposure causes not only an increase in cAMP concentrations but also a decrease in the concentrations of cGMF The time courses of these two events coincide with the minimal stimulus Puration (exposure to 4°C) for eliciting a delayed long term increase of tyrosine hydroxylase activity The data shown in Figure 2 depicts the time constants of the decline of cGMP concentrations and of the simultaneous increase of cAMP concentrations during exposure of the rats to 4°C. As a result of these changes, the ratio of cAMP/cGMP concentrations progressively increases with time; it reaches a maximal value after 24 minutes of exposure to 4℃. The increase of this ratio persists longer than one hour.

Since, as shown in Figure 1, the tyrosine hydroxylase actity of adrenal begins to increase between 12 and 24 hours after cold exposure, we theorize that in the transsynaptically induced long term adaptation of tyrosine hydroxylase, it is possible to distinguish three sequential phases characterized by given biochemical events. The first phase (lasting 1 to 4 hours according to the tissues) is characterized by an increased rate of cholinergic transmitter release and a simultaneous change of the concentration ratios of cAMP and cGMP. The second phase (lasting 8 to 24 hours according to the tissues) has not yet been characterized in terms of a specific biochemical event which is causally related to the long term adaptation of the enzyme. Thoenen et al. have speculated that during this time period the transcription of a new messenger RNA occurs. The third phase of prolonged increase of the enzymatic activity lasty lasts for several days. The length of this phase may depend upon the intensity of the initial stimulus.

Tablel

Tyrosine yhdroxylase Activity (TH) in adrenal glands of rats exposed to 4°c

Hrs At ·4°C	Hrs Elapsed Between Exposure to 4°C and TH Assay	TH nmoles DOPA/hr/gland	
		normal	denervated**
0	24	3·7 ±0.31	3.8 ±0.41
1	23	6.9 ±0.42*	3.6 ±0.38
2	22	7·2 ±0.12*	4·1 ±0.31
4	20	7·2 ±0.41*	———
24	0	8·6 ±0.91*	———
24	24	6.2 ±0.62*	———

*p<0.01 nhen compared with TH of rats at 22°c

**splanchnic nerve was monolaterally severed 5days before the experiments.

Thoenen et al. have repeated similar experiments using two stimuli, namely exposure to 4℃ and repeated swimming stresses in 15℃ water. They have reproduced our finding on the increase of cAMP concentrations in adrenal medulla which lasts for about 60 minutes during exposure of 100 gm rats to 4℃ for 2 hrs. This stimulation was indeed followed by an in tyrosine hydroxylase activity observed at 48 hours after the initiation of 4℃ exposure. We have found an increse of tyrosine hydroxylase activity at both 24 and 48 hours after 1 hour of cold stimulus (Figure 1).

The swimming stress used by Thoenen et al. consists of 3 swimming periods per hour for two hours in 15℃ water. Each period lasts for 5 to 7 minutes. They measured cAMP concentrations of adrenal medulla at various times during the swimming stress and found that the increase of cAMP was much smaller than that bserved during 4℃ exposure. However, tyrosine hydroxylase activity measuredat 48 hours later was increased despite a small increase in cAMP. The results of these experiments seem to argue against a mediating role of cAMP in the delayed increase of tyrosine hyrosine hydroxylase activity, and therefore

prompted us to carry out some studies on the characteristics of the swimming stress in 100 gm rats which included: (a) time course of body temperature after swimming for 7 minutes at 15℃; (b) time course of the concentrations of cAMP and cG MP in adrenal medulla; (c) time course of the changes of cAMP/cGMP concentration ratios in the adrenal; (d) tyrosine hydroxylase activity measured 24 hours later.

The data reported in Figure 3 show: (1) A swimming stress of 7 minutes at 15°C provokes an increase of the cAMP concentrations in innervated adrenal reaching a peak value of 4 to 5 folds of normal values in 30 minutes; the initial rate of this accumulation is reduced when compared to that found in the rats

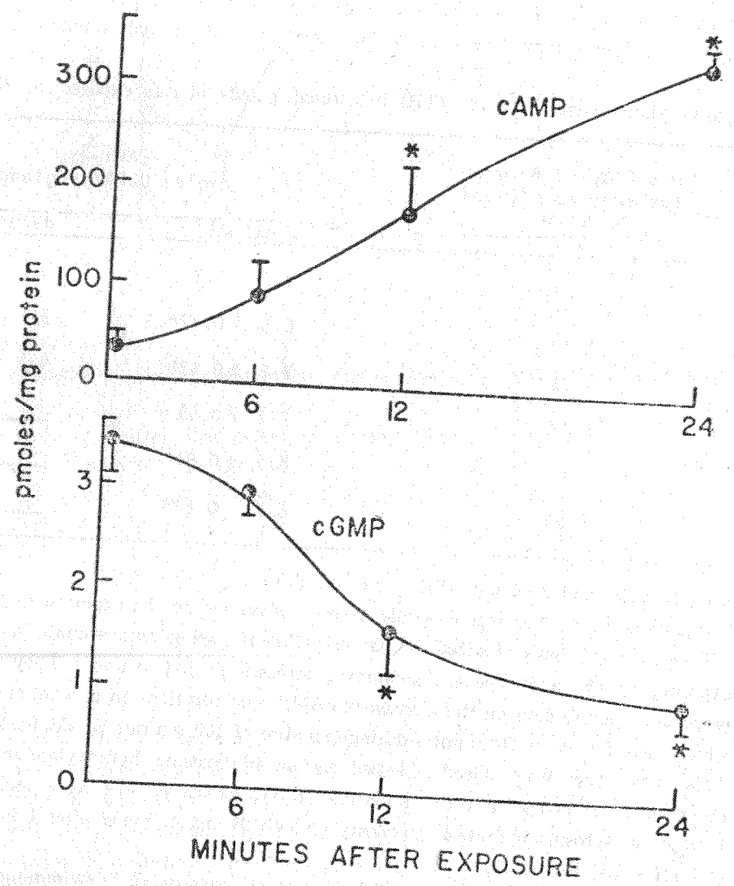

FIGURE 2 Cyclic 3',5'-AMP and cyclic 3',5,-GMP concentrations in adrenal medulla at various times after cold exposure. Adrenal medulla dissected as dissected as described by Guidotti and Costa cAMP and cGMP were separated by the method described by Mao and Guidotti and assayed by activation of two protein kinases (cAMP and cGMP dependent, respectively.)
*P<0.05 when compared with control values.

exposed to 4°C ; denervated adrenal fails to show any change in cAMP concentrations as reported with exposure to 4°C ; (2) the cCMP concentrations are greatly reduced after swimming stress-this reduction reaches its maximum around 30 minutes and gradually returns to normal values over three hours, whereas the concentrations of cGMP in denervated adrenal medulla are not significantly reduced by the swimming stress when they are compared to those of denervated glands of animals kept in normal conditionls-however, the basal cGMP concentr ations are reduced by denervation; (3) the cAMP/cGMP concentration ratios are increased by 15-fold after the swimmin stress and this increase persists for longer thanninety minutes; (4) most strikingly, the body temperature (measured rectally) is greatly reduced at the end of one period of swimming stress (from 37℃ to 22℃) and stays low for about one hour. The tyrosine hydroxylase activity of adrenal gland is increased at 24 hoursafter one swimming stress

Based upon the above findings, we believe that Thoenen et al.'s results do not invalidate the positive relationship between an early change in the cAMP/cGMP concentration ratios and the increase of tyrosine hydroxylase activity proposed by us. It is conceivable that the consequences of the swimming stress are disastrous to the homeostasis of the yound rats, as shown by the remarkable drop in body temperature barely compatible with life. What Thoenen et al. call exhaustion inckudes an extreme hypothermia which is much prolonged when the swimming stress is repeated 3 times in one hour. It is therefore no surprise, and te be expected, that when the body temperature is decreased by 15℃, the cAMP concentration fails to increase. However, we observed that when body temperature returns to higher levels, a dramatic increase in the cAMP/cGMP concentration ratio follows (Figure 3). Consequently, in our point of view the experiments with swimming stress fail to negate the med iating roles of cyclic nucleotides for tyrosine hydroxylase induction. 待續

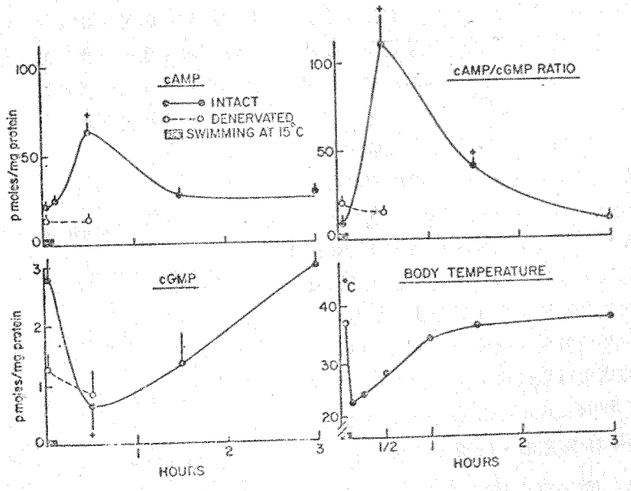

FIGURE 3 Adrenal medullary cAMP and cGMP concentrations, rectal temperature and cAMP/cGMP concentration ratios at various times after swimming stress of 7 minutes at 15℃.
+P<0.05 when compared with control values.