Bull. Inst. Zool., Academia Sinica **16**(2): 131-135 (1977)

A METHOD TO STUDY THE RATE OF INTRASYNAPTOSOMAL CATECHOLAMINE BIOSYNTHESIS

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Received for publication, Nov. 5, 1977

ABSTRACT

Wei-Kung Wang and Yi Chiang (1977). *A method to study the rate of intrasynaptosomal catecholamine biosynthesis.* Bull. Inst. Zool., Academia Sinica **16**(2): 131-135. A new instrument and method to study catecholamine has been constructed. $^{14}CO_2$ from crude synaptosomal preparation utilizing L-(1-^{14}C)tyrosine were measured continuously by a respirometer. This method will be especially useful to study the compartmentation as well as the drug effect on catecholamine biosynthesis.

Catecholamines include the important neurotransmitters, dopamine, norepinephrine and epinephrine. It is generally presumed that tyrosine hydroxylase (T. H.) is the rate-limiting enzyme, therefore the activity of T. H. is usually used as the indicator of the synthetic rate of catecholamine[10,11]. However, recently it has been found that the kinetic properties of rat brain T. H. are significantly altered concomitant with its interaction with nerve ending membrane component[7], and the micro-environment of the nerve ending may also be significantly different from that in the assay solution which contains artificial stimulants to increase the activity.

Recently Weiner[15] used intact peripheral nerve-ending preparation to study the $^{14}CO_2$ liberated from (1-^{14}C)tyrosine as the indicator of the conversion of tyrosine to dopamine and Kuczenski[8] modified this method and applied it to the study of rat nigro-striatal dopaminergic pathway.

In their system the endogenous dihydropteridine was used as the cofactor. However there was no steady oxygen supply to keep the normal metabolism going, and the $^{14}CO_2$ liberation rate was linear only for about 20 min.

Here we have further modified the assay and used the newly designed respirometer to supply the oxygen and continuously measured the $^{14}CO_2$ output from the tissue. The incubation medium was also significantly simplified to mimic the cerebrospinal fluid; this system was more similar to *in vivo* situation compared to the assay condition used by Weiner or Kuczenski, and it gave the metabolic rate every ten minutes interval, instead of the sum of the total metabolic product. It used the 1,000 g supernatant as the crude synaptosomal preparation so as to keep the normal metabolism. This preparation will be especially useful for studing drug effect on catecholamine metabolism.

MATERIALS AND METHODS

Male Sprauge-Dawley rats (Weight 180 g-230 g) raised in our laboratory were sacrificed by decapitation. Brains were removed and dissected on ice. Corpora striata (containing caudate nucleus and a portion of putamen) and hy-

pothalamus were removed and homogenized in 10 vol of 0.32 M sucrose by Teflon pestle tissue homogenizer with 0.25 mm clearance (from Arthur H. Thomas Co., Philadelphia). Homogenates were centrifuged (0°-4°) at 1,000 g for 15 minutes to sediment nuclei and debris. Several 50 μl portions of the supernatant (equivalent to 5 mg wet tissue) containing synaptosomes and other cellular component were added to test tubes each containing 150 μl of physiological medium which contained 125 mM NaCl, 1.48 mM $CaCl_2$, 4.8 mM KCl, 2.5 mM $MgSO_4$, 22 mM NaH_2PO_4 10 mM $NaHCO_3$ and 16 mM glucose (chemicals are from Sigma) and gave a final pH of 6.6 when equilibrated with 95% O_2-5% CO_2 gas. Tyrosine concentration was 5 μM (specific activity 50 m Ci/m mole from New England Nuclear Corp.).

The respriometer (detecter and counting system is from Canberra Inc. Conn.) is shown in Fig. 1. The $^{14}CO_2$ output was measured in every ten minutes. The incubation temperature was 37.5°.

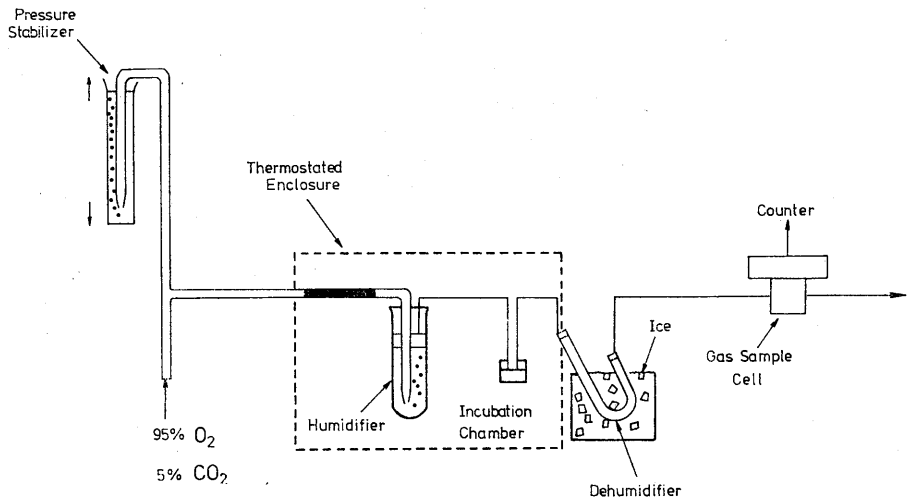

Fig. 1. Respirometer used to measure $^{14}CO_2$ that is released from the tissue in the incubation chamber continuously.

RESULTS

In striatal preparation, the rate of $^{14}CO_2$ output from (1-^{14}C)-tyrosine continuously increased for about 50-60 minutes. It reached a maximum and then decreased slowly. The rate of reaction depended on the pH value of the incubation medium and the freshness of the preparation. A group of typical data are shown in Fig. 2; the activity is not very sensitive to pH value, however it does show a maximum around pH$\simeq$6.6. This is similar to the results of Kuczensky[8]. The activity reduced slowly on prolonged incubation. The maximum rate was reduced very slowly in the aging of the preparation. The maximum rate was about 0.2 p mole min^{-1} mg^{-1}. This is also very similar to the results of Kuczensky; he used 20 μM (50 m Ci/m mole) tyrosine in the incubation medium and got maximum CO_2 evolution rate of 0.8 p mole min^{-1} mg^{-1} for Caudate-putamen. The area distribution was also studied by the present method. There were also high activity at cerebral and cerebellum cortex. The maximum rate of CO_2 liberation from tyrosine under similar incubation was about $\frac{1}{4}$ to $\frac{1}{5}$ of that of striatal and showed a similar pH dependence.

The hypotonic preparation which used distilled water to homogenize the striatal tissue (sucrose were added afterward, the incubation medium were exactly the same) showed activity about 1/6 of that the standard preparation.

Unfortunately, neither Kuczensky[8] nor Harris[3] had presented this type of results and no comparison could be made. To explain the exponential increase of the curve, a model has been constructed.

MODEL

This model was first derived for glucose metabolism[14]. Here a simplified version is given.

In a metabolic pathway, if we look at one specific intermediate α with pool size P_1, the flux rate into P_1 is V_α. The flux rate out of P_1 is V_β. In a steady state $V_\alpha = V_\beta = V$. Suppose the flux V into P_1 contain isotope ^{14}C and has specific activity S_α, the specific activity in the P_1 is zero at $t=0$. When the intermediate α is converted at rate V and flows into the pool P_1, the rate change of specific activity S in P_1 is

$$\frac{dS}{dt} = \frac{V(S_\alpha - S)}{P_1}$$

This equation has solution $S = S_\alpha(1 - e^{-\frac{V}{P_1}t})$. For more than two intermediates, the specific activity of the product will be $S = S_\alpha(1 - e^{-\frac{V}{P_i}t})$, where P_i is the largest pool in the series of reaction.

This model is therefore especially useful in studying the rate-limiting step, which usually has a large pool and a low metabolic rate. The enzyme at this step is controlling the metabolic rate of the whole metabolic pathway. The subsequent intermedaite will have the same rate as this one and the pool size will be much smaller. Therefore if we measure the $\tau (= P_i/V$ for multiple intermediate system) for the specific activity increase at the rate-limiting step or any intermediate after the rate-limiting step, V at the steady state can be obtained by extrapolating the specific activity to the maximum, we may thus find the pool size of the intermediate that is the substrate for the rate limiting enzyme.

In the present case the rate-limiting enzyme is T. H.. Therefore, what we studied will be the activity of tyrosine hydroxylase in the synaptosomes, and the pool we figure out by the equation will be the pool of intrasynaptosomal tyrosine. Both the activity and the pool size may be affected by drug administration.

DISCUSSION

The results in Fig. 2 does follow an exponential curve to increase, however it does not stay at the maximum rate as predicted by the model. The discrepancy may be due to the product inhibition that has not been considered in the model.

In this preparation, there is no stimulation to release catecholamine, the product, so that it will be accumulated in the synaptosome. It is well known that dopamine and norepinephrine will effectively inhibit the activity of T. H.[10]

Another possibility for the decrease of metabolic rate is the tissue death. Some study on general metabolism will be needed to clarify this.

It has long been suggested that newly synthesized catecholamines are released in preference to stored catecholamines during the activation of aminergic neurones[6,12]. Some authors also suggested that this effect is due to different function of soluble and particulate T. H.[7,9]. The results from this method, which are designed to study compartmentation, has not find sign of two separate pools or two different kinds of enzyme. Some studies with electrical or drug stimulation that will release the catecholamine[4, 5,16] are underway to further investigate this possibility. Some study on the effect of tyrosine concentration is also underway to see if the activity-time curve (Fig. 2) can be significantly changed; this will be the evidence that at least two forms of enzyme with different Km are participating in the metabolism.

This method will be especially useful for pharmacological studies. It may study the catecholamine biosynthesis in similar condition by incubating the tissue slice while it does not have the problem of substrate diffusion. We have been looking for the effect of acetylcholine and choline on the catecholamine biosynthesis. Some preliminary results show that both drugs stimul-

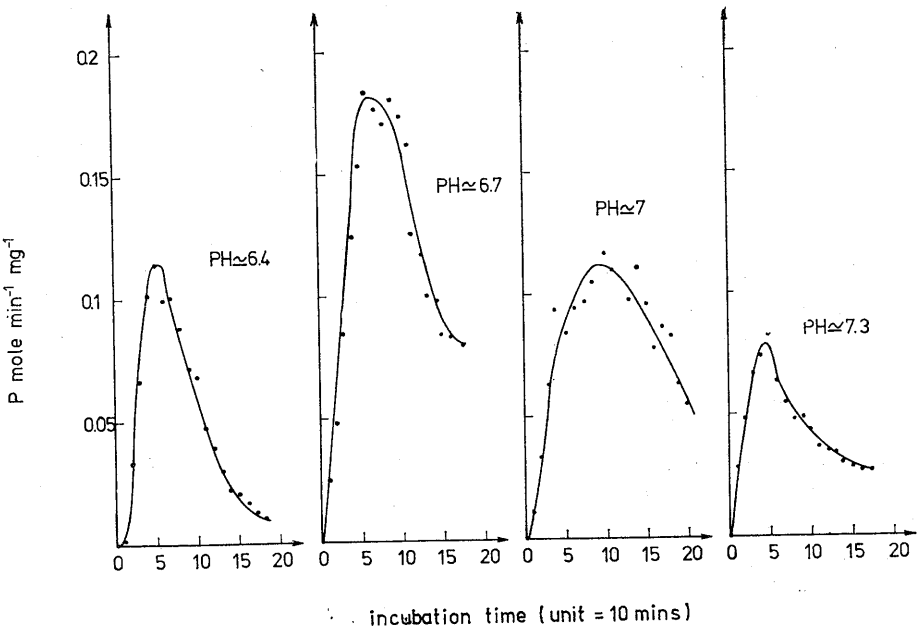

Fig. 2. Catecholamine biosynathetic rate as the function of time and pH.

ate catecholamine biosynthesis. This implies that the stimulating effect is not totally due to a polysynaptic mechanism as suggested by many authors[1,13].

This method may also be used for *in vivo* study similar to the experiments performed by Glowinski[2] who used ^3H_2O as the indicator for catecholamine synthesis.

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一種測量氨基苯二酚生理合成速率之方法

王唯工　蔣　宜

本篇報告連續測量神經扣結中 L-(1-^{14}C) 酪氨酸代謝所產生之 $^{14}CO_2$ 一種新儀器及方法，此法對氨基苯二酚生理合成之分室及藥物效應特別方便。