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DIFFERENT INHIBITORY EFFECTS OF DOPA, DOPAMINE AND APOMORPHINE ON DOPAMINE BIOSYNTHESIS

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Wei-Kung Wang, Tsung-Yung Hai and YiChiang (1980) Different inhibitory effects of dopa, dopamine and apomorphine on dopamine biosynthesis. *Bull. Inst. Zool., Academia Sinica* **19**(1): 33-37. Inhibitory effects of dopa, dopamine and apomorphine on dopamine synthesis were studied. Drugs were added into crude synaptosomal preparations with L(1-^{14}C)-Tyrosine, and liberated $^{14}CO_2$ were measured for every 10 minutes. Special attention was paid to the time course of $^{14}CO_2$ liberation. The inhibitory effect of dopa on $^{14}CO_2$ liberation occurred about 40 minutes after administration *in vitro*, while the effects of dopamine and apomorphine occurred quickly. The different inhibition pattern indicates that dopamine and apomorphine inhibit the dopamine biosynthesis through a different mechanism from that of dopa.

Presynaptic autoregulation on norepinephrine release has received increasing amount of attention. There is now good evidence that the release of norepinephrine is modulated by a direct feedback mechanism, the neurotransmitter present in the synapse activate the presynaptic receptor and then depress the depolarization-induced release of the amine[2~4]. However, a similar mechanism of presynaptic autoreceptor controlling dopamine (DA) release is still an open question[1]. Some authors suggested an indirect mechanism in controlling the DA release, the activated receptor will inhibit the synthesis of amine first and then decrease its release. The decrease in the release of amine is, therefore, a consequence of blockaded synthesis. To distinguish between the direct and indirect mechanisms is a difficult problem, because (a) in the direct mechanism, the decreased release of DA will result in the increased amount of DA accumulated in the presynaptic terminal, and through the product inhibition, the accumulated DA will inhibit the activity of tyrosine hydroxylase (T. H.), (b) dopamine may also be taken up by presynaptic terminal and inhibit the DA synthesis, the ultimate result in terms of T. H. activity is the same. Both direct and indirect mechanism will decrease the activity of T. H.

In the present study, we took advantage of the newly designed instrument to study the rate of dopamine synthesis. Inhibitory effects of DA, dopa, and apomorphine on DA synthesis were studied. Special attention was paid to the time course of DA synthetic rate and tried to distinguish whether the decreased synthetic activity was directly due to the presynaptic auto-receptor modulation or an indirect result of product inhibition.

MATERIALS AND METHODS

Male Sprauge-Dawley Rat (Weight 180 g-230 g) raised in our laboratory was sacrificed by decapitation. Brain was removed and dissected on ice. Corpora striatum (containing caudate nucleus and a portion of putaman) was removed and homogenized in 10 volumes of ice-cold 0.32 M sucrose by teflon pestle tissue homogenizer with 0.25mm clearance

(Arthus H. Thomas Co., Philadelphia). The homogenate was centrifuged (0°-4°C) at 1,000×g 15 minutes to sediment nuclei and debris. Several 50 $\mu\ell$ aliquots of the supernatant (equivalent to 5 mg wet tissue) containing synaptosomes and other cellular components were added to test tubes each containing 150 $\mu\ell$ 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 mM (specific activity 50 m Ci/m mole from New England Nuclear Corp.). Dopa, dopamine or apomorphine were added to the incubation medium with 10 $\mu\ell$ 0.1N phosphorus buffer PH=6.6 as carrier. The control tube was also added with 10 $\mu\ell$ of the same buffer. Liberated $^{14}CO_2$ from L(1−^{14}C)tyrosine were measured for every 10 minutes by a respirometer[7].

RESULTS

The effects of apomorphine on dopamine synthesis of striatal preparation were shown in Fig. 1. The inhibitory effect increased with the increasing concentration of apomorphine. When the apomorphine concentration was 5×10^{-7} M, nearly no inhibitory effect could be seen. The synthetic rate was about 83% in comparison with that of control group. As apomorphine concentrations was increased to 1×10^{-6}, 5×10^{-6}, 1×10^{-4} M, the dopamine synthetic rate was 78%, 45%, 17% of that of control group respectively. The synthetic rates were calculated by averaging over six to ten steady state values after 50 minutes of incubation.

The $^{14}CO_2$ liberation rate in presence of 1×10^{-5} M dopa was given in Fig. 2. The amount of liberated $^{14}CO_2$ was not significantly inhibited untill 50 minutes after in vitro administration of dopa (M±SE were not overlapping after 50 minutes of incubation). The inhibitory effect from 50 to 120 minutes was about 26%. Fig. 3 indicated that when 1×10^{-5} M dopamine was added to striatal preparation, the rate of $^{14}CO_2$ liberation was inhibited instantly. It should be noted that the inhibition pattern was similar to that of apomorphine at concentration of 5×10^{-6} M, and the time courses of $^{14}CO_2$ liberation in presence of dopa or dopamine were quite different. The inhibitory effect of dopamine from 50-120 minutes after addition was

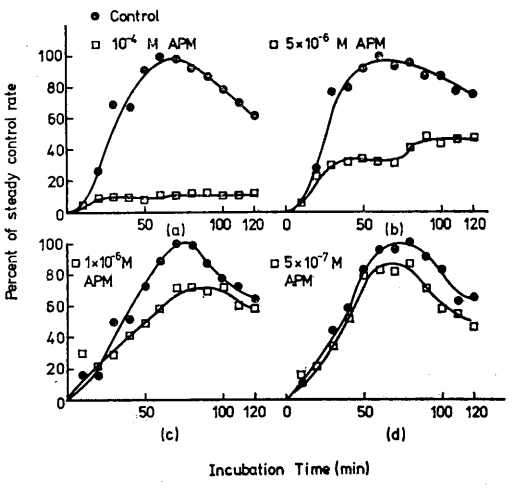

Fig. 1. Effects of apomorphine (APM) on dopamine synthesis vs a function of incubation time.

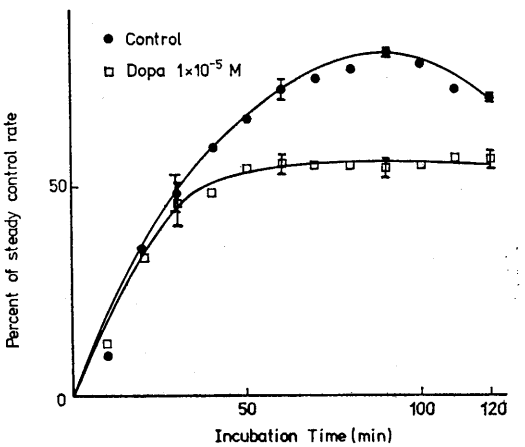

Fig. 2. Effect of dopa on $^{14}CO_2$ liberation rate, each point represents the mean of four experimental values. At t=30 min, 60 min, 90 miu, 120 min the standard errors were shown by I.

about 50%. We have also observed the inhibitory effects of dopa and DA at concentration of 1×10^{-6} M. As shown in Fig. 4, the $^{14}CO_2$ liberation rate was slightly inhibited by dopa (6%) and moderately blockaded by DA (18%).

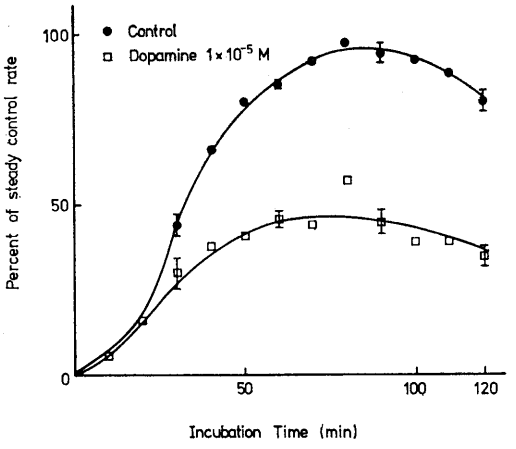

Fig. 3. Effect of dopamine on $^{14}CO_2$ liberation rate, each point represents the mean of four experimental values. At t=30 min, 60 min, 90 min, 120 min, the standard errors were shown by I.

DISCUSSION

Before further discussion of the result, we shall first explain briefly the theory on which we could distinguish some different mechanisms on the modification of $^{14}CO_2$ liberation rate. When we use a labelled precursor to study the formation of certain compound in a separated compartment, for example, in a cell or a synaptosome, the most difficult point is how to determine the concentration of the endogenous precursor. If we measured the formation of the labelled product after a specific reaction time, the change of specific activity due to the dilution of the endogenous precusor will greatly affect the final result.

Here we will derive a mathematical model to simulate the production pattern of our labelled compound from the preparation and try to explain the advantage of our approach.

This mathematical model was first derived for glucose metabolism[6]. Here a simplified version will be given. In a metabolic pathway, if we look at a specific intermediate α with endogenous pool size P_1, the flux rate into P_1 is V_α, and the flux rate out of P_1 is V_β. In a

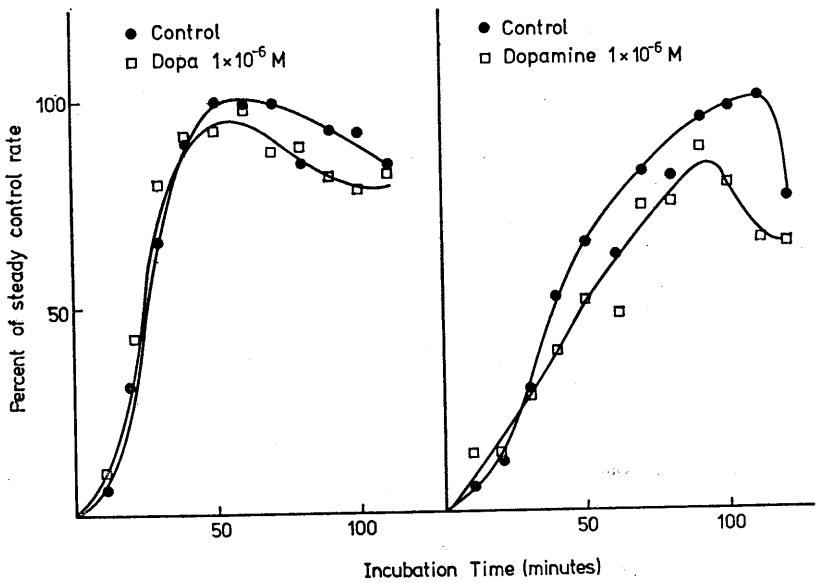

Fig. 4. Effect of dopa and dopamine on $^{14}CO_2$ liberation at lower concentration.

steady state $V_\alpha = V_\beta = V$. Suppose the flux into P_1 contains isotope ^{14}C of 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 flow into the pool P_1, the rate of change of specific activity S in P_1 is

$$\frac{dS}{dt} = \frac{V(S_\alpha - S)}{P_1} \qquad (1)$$

This equation has a solution

$$S = S_\alpha(1 - e^{-\frac{V}{P_1}t}) \qquad (2)$$

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

According to equ. (2), the amount of $^{14}CO_2$ produced from the following reactions

$$(1-^{14}C)\text{tyrosine} \rightarrow (1-^{14}C)\text{dopa} \rightarrow \text{dopamine} + {}^{14}CO_2$$

inside the synaptosome will follow a exponentially increasing curve and reach steady state with time constant P/V, where V is metabolic rate of this series of reaction, while P is the pool size of tyrosine inside the synaptosome.

There are several mechanisms that may reduce the liberated $^{14}CO_2$ without directly affecting T. H. and these mechanisms will not be able to detect by direct enzyme activity study.

(1) If there is uptake of cold dopa into the synaptosome, the specific activity of the dopa inside the synaptosome will be reduced, thus reduce the liberated $^{14}CO_2$.

(2) If there is uptake of dopamine into the synaptosome, the T. H. activity will be reduced so are the liberated $^{14}CO_2$.

(3) If the pre-synaptic dopamine autoreceptor is stimulated, two possible mechanisms have been suggested: (a) the release of dopamine will be reduced. (b) the synthesis of dopamine will be reduced[5].

For mechanism (a) the dopamine will accumulate in the synaptosome and inhibit T. H., the V of the dopamine formation will be reduced, so will the liberated $^{14}CO_2$. This mechanism is similar to the mechanism (2).

For mechanism (b), the V will be directly affected and thus reduce the $^{14}CO_2$ liberation.

In our experiment, we tried to distinguish between the mechanisms (1), (2), as well as (3a) which are secondary effects of a slow accumulation of dopa or dopamine and (3b) which is a direct effect and is supposed to happen with a very short time delay.

The time course of $^{14}CO_2$ liberation when incubated with dopa clearly indicates that the effect of dopa on reducing $^{14}CO_2$ release does need some time to occur. This result suggests that the inhibitory effect of dopa was due to presynaptic uptake of dopa. Apomorphine, a substance which appears to stimulate dopamine receptor, inhibited dopamine synthesis immediately (within the resolution limit of the instrument) after in vitro administration. This fact elucidates that the presynaptic receptor activated by apomorphine inhibits dopamine synthesis instantly and any effect on releases seems to be secondary to that on synthesis.

As pointed out in the result that the inhibitory pattern of dopamine was similar to that of apomorphine, therefore, we could infer that the dopamine in synapse could regulate its release by the same way as apomorphine, and the indirect modulation of release may be a secondary effect.

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Dopa, Dopamine 及 Apomorphine 對 Dopamine 生合成的不同抑制作用

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本篇研究多巴 (dopa)、多巴敏 (dopamine)，及阿朴嗎啡 (apomorphine) 對多巴敏合成之影響。以藥物影響神經扣結中 L-(1-^{14}C) 酪氨酸所釋出之 $^{14}CO_2$ 作爲指標，尤其注意 $^{14}CO_2$ 釋出量隨加藥時間之改變，多巴於加藥後約 50 分鐘開始抑制 $^{14}CO_2$ 之釋出，而多巴敏及阿朴嗎啡之效果皆迅速發生，此結果顯示多巴敏，阿朴嗎啡與多巴在結前之作用不同。