Study On the Mechanism of Dopamine-Induced Inhibition of Hemoglobin Synthesis in Chick Blastoderm *in Vitro*[1] (35149)

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Dopamine is essential for the function of adult nervous tissue (1). However, its significance in the non-nervous tissue has only been recently realized (2–8). Dopamine given before X-ray exposure protects whole-body irradiated rodents against lethality and irradiated DNA solution against base damage (2–4). Dopamine reduced mouse spleen DNA synthesis *in vivo* and the growth of HeLa cell population *in vitro* (5). Dopamine causes hyperglycemia in mice probably by interfering with the release of insulin (6). More recently, we have reported that dopamine inhibits hemoglobin synthesis in chick blastoderm *in vitro* without affecting the development of the embryo proper (7). Other catecholamines (norepinephrine and epinephrine) and metabolites of dopamine (homovanillic acid and hydroxyindoleacetic acid) do not produce such an effect (8). These agents at a dose comparable to dopamine are lethal to the blastoderms and at a nonlethal dose level have no effect on hemoglobin synthesis. Serotonin at a dose comparable to dopamine has no effect on the development of chick blastoderm *in vitro*.

This communication describes some new experiments on the mechanism of dopamine-induced inhibition of hemoglobin synthesis in chick blastoderm *in vitro*.

Materials and Methods. Chemical and Radioisotopes. Dopamine (3, 4-dihydroxyphenylethylamine, HCl, Nutritional Biochemicals, Co.) δ-aminolevulinic acid, HCl (K & K Laboratory) were obtained from the commercial sources. L-Leucine-^{14}C(U) (sp act 10 mCi/mmole) and 5-aminolevulinic acid-4-^{14}C hydrochloride (sp act 53 mCi/mmole) were obtained from Amersham/Searle.

Media. The semisolid culture medium of Ringer-Agar and egg extract was prepared in the manner described by Spratt and Hass (9); however, the pH of the final medium was reduced to 6.7–6.8 by the addition of 0.1 *M* sodium phosphate (monobasic) solution (pH 4.6) to reduce the auto-oxidation of dopamine. Dopamine or δ-aminolevulinic acid (ALA) was dissolved in 0.1 *M* sodium phosphate solution before its addition to the constituents of the culture medium. The control medium had an equivalent volume of 0.1 *M* sodium phosphate solution. Egg yolk of the extract does not contain endogenous dopamine but has a small amount of norepinephrine and epinephrine (10). Therefore, the culture medium used in this investigation had no endogenous dopamine.

To study if dopamine-induced inhibition of hemoglobin synthesis in chick blastoderm *in vitro* is due to a reduction in protein synthesis, the incorporation of ^{14}C-leucine into TCA-insoluble protein was measured. The incorporation of ^{14}C-aminolevulinic acid (ALA) into hot TCA insoluble protein has been considered as a sensitive measurement of hemoglobin synthesis (11). If dopamine inhibits hemoglobin synthesis by reducing the incorporation of ALA into heme, it should reflect in the reduction of ^{14}C-ALA incorporation into protein. ^{14}C-leucine or ^{14}C-ALA was added to the dopamine-treated (500 μg/ml) and control medium at a concentration of 20 μCi/60 ml. Blastoderms of six to eight somites were transplanted in the dopamine-treated and control medium and incubated at 38.5° for 24 hr. After incubation each blastoderm was removed from the medium, rinsed in Ringer–saline to remove egg yolk and homogenized in 2 ml of distilled water.

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TABLE I. Incorporation of ^{14}C-Leucine and ^{14}C-5-Aminolevulinic Acid (ALA) into Protein of Chick Blastoderm *in Vitro*.[a]

Treatment	Radioactive precursor	(cpm/mg × 10^3)	Total protein/blastoderm (mg)
Control (10)	^{14}C-leucine	5.6 ± 1.3[b]	5.5 ± 1.7
Dopamine (12)	^{14}C-leucine	4.7 ± 0.9	4.6 ± 1.5
Control (7)	^{14}C-ALA	2.8 ± 0.7	
Dopamine (7)	^{14}C-ALA	3.4 ± 1.3	

[a] ^{14}C-Leucine or ^{14}C-ALA was added to the dopamine-treated (500 μg/ml) and control medium at a concentration of 20 μCi/60 ml. Blastoderms of six to eight somites were transplanted to the dopamine-treated and control medium. After 24 hr of incubation at 38.5° the incorporation of ^{14}C leucine or ^{14}C-ALA into protein fraction of dopamine-treated and control blastoderms was measured. The total protein per blastoderm was also determined.

[b] Standard deviation.

The protein was extracted by Siekevitz's method (12). The protein was dissolved in 2 ml of 2 *N* NaOH and 0.2 ml was added to each counting vial containing 10 ml of dioxane–solvent system for assaying radioactivity (13). Another aliquot was taken to determine the amount of protein by the method of Lowry *et al.* (14).

To measure the amount of ^{14}C-ALA into protein, the protein precipitate (after being washed in 5% TCA) was boiled in 5% TCA at 90° for 15 min and then washed once with water to remove TCA. The washing of precipitate with organic solvent was avoided to reduce the extraction of heme. The washed protein was dissolved in 2 ml of 2 *N* NaOH with slight warming. Radioactivity was assayed by a liquid scintillation β-spectrometer, using an external standard. The amount of quenching for each sample was similar and therefore no correction for this factor was made. Data were expressed as counts per minute per milligram of protein.

To study if the concentration of ALA is a limiting factor in the dopamine-induced inhibition of hemoglobin synthesis in chick blastoderm *in vitro,* the effect of exogenous ALA was studied. Blastoderms were transplanted to the four groups of medium (control medium; medum containing 500 μg/ml of dopamine and 170 μg/ml of ALA; and medium containing 170 μg/ml of ALA). After 24 hr of incubation each blastoderm was removed, homogenized in 0.1 *M* NaCl and the amount of hemoglobin was determined according to the method of Hanks *et al.* (15). Since dopamine inhibits hemoglobin synthesis, it was necessary to pool two blastoderms for the hemoglobin analysis. The amount of hemoglobin (μg/blastoderm) was determined in control and experimental groups. In each experiment, the hemoglobin value in experimental blastoderms was expressed as percentage of controls.

Results and Discussion. Table I shows that the incorporation of ^{14}C-leucine into TCA-insoluble protein was similar in dopamine-treated and control blastoderms indicating that dopamine inhibited hemoglobin synthesis without affecting the total protein synthesis. The amount of protein per blastoderm was also similar in dopamine-treated and control blastoderms showing that dopamine caused no significant cellular loss: If dopamine specifically inhibits globin synthesis, it is possible that this effect may be masked when one measures the synthesis of total protein.

Dopamine may reduce hemoglobin synthesis by interfering with the synthesis of heme. The incorporation of ^{14}C-ALA, a precursor of heme, into TCA-insoluble protein has been considered as a sensitive index of hemoglobin synthesis (11). This paper shows that the incorporation of ^{14}C-ALA into TCA insoluble protein was similar in both the dopamine-treated and control blastoderms indicating that the mechanism of incorporation of ALA into heme was not damaged by dopamine (Table I).

Dopamine may reduce the endogenous concentration of ALA by inhibiting the activ-

TABLE II. Effect of Dopamine and ALA on Hemoglobin Synthesis of Chick Blastoderm *in Vitro*.[a]

No. of blastoderms	Treatment	Hemoglobin/blastoderm (% of controls)
10	Dopamine	25.0 ± 12.0[b]
16	ALA (170 μg/ml)	79.0 ± 15.0
10	ALA (170 μg/ml) + dopamine	16.0 ± 6.0
6	ALA (50 μg/ml)	123 ± 21.0
8	ALA (50 μg/ml) + dopamine	38 ± 17.0

[a] Chick blastoderm of six to eight somites were incubated in the medium containing dopamine (500 μg/ml), dopamine (500 μg/ml) plus ALA (170 μg/ml or 50 μg/ml), ALA (170 μg/ml or 50 μg/ml) or equivalent volume of sodium phosphate solution. After 24 hr of incubation the amount of hemoglobin (μg/blastoderm) was determined by the benzidine method as modified by Hanks *et al.* (15). In each experiment the hemoglobin value of experimental blastoderms was expressed as percentage of controls. The average hemoglobin value of 18 control blastoderms from six experiments was 41.0 ± 10.0 μg/blastoderm.

[b] Standard deviation.

ity and/or synthesis of ALA synthetase, a rate-limiting enzyme in the biosynthesis of heme. Therefore, the effect of exogenous ALA on dopamine-treated blastoderms was studied. ALA (170 μg/ml) has been shown to stimulate hemoglobin synthesis in chick blastoderm *in vitro*, provided blastoderms of six to seven somites are assayed only 4 hr after incubation (16). In our experiments, the hemoglobin was analyzed 24 hr after incubation. ALA (170 μg/ml) did not stimulate hemoglobin synthesis in chick blastoderms of six to eight somites, rather it produced a slight reduction in hemoglobin formation. This was further demonstrated by the fact that dopamine in combination with ALA inhibited the hemoglobin synthesis slightly more than by dopamine alone (Table II). Since the medium containing dopamine and ALA showed less auto-oxidation of dopamine than that containing only dopamine, the addition of ALA may have lowered the pH of the medium and thereby increases the effective dose of dopamine to the blastoderms. Indeed, when ALA was added, the pH of the sodium phosphate solution was dropped from 4.6 to 3.8. In order to show if the reduction in pH of the medium contributed to the lowering of hemoglobin synthesis further, the pH of ALA solution was adjusted to 4.6 before its addition to the constituents of the medium. Results showed that a slight inhibition of hemoglobin synthesis produced by ALA was not related to the lowering of pH of the medium. Therefore, ALA at a dose of 170 μg/ml may have slight inhibitory effect on the hemoglobin synthesis. To study the effect of ALA further, a lower dose of ALA (50 μg/ml) was used. The amount of hemoglobin in blastoderm treated with dopamine alone or in combination with ALA was similar (Table II). Thus ALA did not reverse the dopamine-effect on hemoglobin synthesis.

This paper shows that dopamine-induced inhibition of hemoglobin synthesis in chick blastoderm *in vitro* is not related to the rate or the amount of total protein synthesis. If dopamine reduces heme synthesis, this is not due to the reduction of ALA concentrations or the impairment of ALA incorporation into heme. Dopamine may be acting on some other sites of heme biosynthesis. A number of investigators have shown that the inhibitors of protein and RNA synthesis reduce the formation of hemoglobin in chick blastoderms *in vitro* (17–22). The exact mechanism of dopamine-induced reduction of hemoglobin synthesis remains obscure.

Human neuroblastoma tumors are characterized by a rapid synthesis of dopamine and its precursor, dopa (23, 24). We are speculating that if dopamine also inhibits hemoglobin synthesis in humans, certain degree of anemia should be present in these patients.

Summary. The mechanism of dopamine-induced inhibition of hemoglobin synthesis in chick blastoderm *in vitro* was investigated. Dopamine did not reduce the incorporation of ^{14}C-leucine or ^{14}C-aminolevulinic acid (ALA) into protein. The total protein per blastoderm was similar in both the dopamine-treated and control blastoderms. Addition of exogenous ALA to the dopamine-treated medium did not reverse the effect of dopamine on hemoglobin synthesis.

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1. Hornykiewicz, O., Pharmacol. Rev. **18**, 925 (1966).

2. Prasad, K. N., and VanWoert, M. H., Science **155**, 470 (1967).

3. Prasad, K. N., and VanWoert, M. H., Radiat. Res. **37**, 305 (1969).

4. Prasad, K. N., and VanWoert, M. H., Int. J. Radiat. Biol. **14**, 79 (1968).

5. Prasad, K. N., and Kollmorgen, G. M., Proc. Soc. Exp. Biol., Med. **132**, 426 (1969).

6. Hakanson, R., Lundquist, I., and Rerup, C., Eur. J. Pharmacol. **1**, 114 (1967).

7. Prasad, K. N., and Seale, R. U., Proc. Soc. Exp. Biol. Med. **131**, 1308 (1969).

8. Prasad, K. N., Seale, R. U., and Kollmorgen, G. M., J. Cell. Biol. **43**, 107 (1969) (abstr).

9. Spratt, N. T., Jr., and Hass, H., J. Exp. Zool. **144**, 139 (1960).

10. Ignarro, L. J., and Shideman, F. E., J. Pharmcol. Exp. Ther. **159**, 38 and 49 (1968).

11. Fantoni, A., Chapelle, A. De La, and Marks, P. A., J. Biol. Chem. **244**, 675 (1969).

12. Siekevitz, P., J. Biol. Chem. **195**, 549 (1952).

13. Bray, G. A., Anal. Biochem. **1**, 279 (1960).

14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

15. Hanks, G. E., Cassel, M., Ray, R. N., and Chaplin, H., J. Lab. Clin. Med. **56**, 486 (1960).

16. Wainwright, S. D., and Wainwright, L. K., Can. J. Biochem. **47**, 1089 (1969).

17. Wilt, F. H., Biochem. Biophys. Res. Commun. **33**, 113 (1968).

18. O'Brien, B. R. A., J. Embryol. Exp. Morphol. **9**, 202 (1961).

19. Deucher, E., and Dryland, A. M. L., Nature (London) **201**, 832 (1964).

20. Wilt, F. H., J. Mol. Biol. **12**, 331 (1965).

21. Levere, R. D., and Granick, S., Proc. Nat. Acad. Sci. U.S.A. **54**, 134 (1965).

22. Wainwright, S. D., and Wainwright, L. K., Can. J. Biochem. **45**, 344, 1648 and 1483 (1967).

23. Mason, G. A., Hart-Mercer, J., Miller, E. J., Strang, L. B., and Wynne, N. A., Lancet **2**, 322 (1957).

24. Kaser, H., Pharmacol. Rev. **18**, 659 (1966).

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