Effect of Dopamine on Hemoglobin Synthesis of Chick Blastoderm *in Vitro** (34094)

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The pharmacological activities of dopamine have been known (1, 2) for many years; however, the physiological significance of dopamine in nerve tissue function has been realized only recently (3–5). More recent evidence suggests that in addition to its role in nervous tissue function, dopamine might influence the activity of non-nervous tissue Dopamine given before X-ray exposure protects whole-body irradiated rodents against lethality and irradiated DNA solution against base damage (6–8). Dopamine also inhibits mouse spleen DNA synthesis *in vivo* and the growth of HeLa cell poulations *in vitro* (9, 10). This communication describes the effects of dopamine on hemoglobin formation in 24-hr chick blastoderm *in vitro*. The effects of dopamine are compared with those of dopa, norepinephrine, epinephrine, and serotonin.

Materials and Methods. Chemicals and radioisotopes. Dopamine (3, 4-dihydroxyphenylethyl amine, HCl, Nutritional Biochemicals Co.), L-dopa (L-dihydroxyphenylalanine, Nutritional Biochemicals Co.), 1-norepinephrine (Levophed bitartrate, 2 mg/ml, Winthrop Laboratories), epinephrine (adrenalin chloride, 1 mg/ml, Park Davis), and serotonin (serotonin creatinine sulfate monohydrate, Regis Chemical Co.) were obtained from the commercial sources. ^{3}H Dopamine (3, 4-dihydroxyphenylethyl amine $^{3}H(G)HBR$, sp act 33.3 mCi/mg) was received from New England Nuclear Co. Ferrous citrate ^{59}Fe (sp act, 5.99 mCi/mg) was obtained from Abbott.

Media. The semisolid culture medium of Ringer-agar and egg extract was prepared in the manner described by Spratt and Hass (11); however, the pH of the final medium was reduced to 6.7–6.8 by the addition of 0.1 *M* phosphate buffer to reduce the autooxidation of compounds under investigation. Dopamine, dopa, or serotonin was dissolved in this buffer before its addition to the constituents of the culture medium. The solution of norepinephrine or epinephrine was added directly to the medium. Egg yolk of the extract does not contain endogenous dopamine but has a small amount of norepinephrine and epinephrine (12). Therefore, the culture medium used in this investigation had no endogenous dopamine.

Transplantation of blastoderm and incubation. Fertile Hyline 934 eggs from commercial sources were received within 24 hr after laying and stored at 12.5° until incubated. After 23–26 hr of incubation, the blastoderms were removed from the yolk and vitelline membrane and staged according to staging system of Hamburger-Hamilton (13). A total of 400 blastoderms of stage 6 or 7 was transplanted to the culture medium containing varying concentrations of dopamine, dopa, norepinephrine, epinephrine, or serotoin. Control bastroderms were transplanted to the medium which had an equivalent volume of the buffer. Transplanted blastoderms were incubated at 38.5° for 24 hr and then examined.

Features of blastoderm of stages 6 or 7. The area vasculosae of these blastoderms have undifferentiated erythropoietic elements, as evidenced by the absence of hemoglobin synthesis. It should be pointed out that when blastoderms of stage 6 or 7 are incubated for a period of 24 hr, only the

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differentiation of erythropoietic elements and not of white blood elements occurs in the area vasculosa. The embryo of blastoderms of stage 6 or 7 is represented by head process and neural folds. The dopamine pathway is not yet developed in these embryos (12).

^3H dopamine and ^{59}Fe uptake studies. ^3H dopamine was added to the control culture medium (1 μCi/ml). Blastoderms of stage 6 or 7 were transplanted to this medium and incubated. Embryo and area vasculosa were separated from each other, washed twice in cold saline, once in 0.01% nonradioactive dopamine solution and then transferred into counting vials containing 0.5 ml of NCS solubilizer. After overnight exposure to the solubilizer, 10 ml of dioxane-solvent system were added to each counting vial. ^3H activity in the embryo and the area vasculosa was assayed by a liquid scintillation β spectrometer, using an external standard to estimate the quenching. The amount of quenching for each sample was similar and therefore no correction for this factor was made.

^{59}Fe was added to the culture medium (0.33 μCi/ml) together with dopamine (50, 500, 1000 μg/ml). After 24 hr of incubation, the blastoderms in the medium were fixed with 10% buffered formalin and the embryos were removed 4 hr later. The area vasculosae were washed twice with distilled water and radioactivity was assayed by an automatic well-type γ scintillation detector.

Results. Effect of dopamine. The effect of dopamine (10, 50, 200, 500, 1000 μg/ml) on the area vasculosa was observed by direct microscopy in the living blastoderm. After 24 hr of incubation, control blastoderms and blastoderms cultured in the dopamine-treated medium (10–200 μg/ml) had well-formed vascular channels with circulating, hemoglobin-rich embryonic erythroblasts. At a higher concentration (500 μg/ml), very little hemoglobin was evident and the vascular channels of the area vasculosa were greatly reduced in size and number; however, the development of the embryo was comparable to the control. Dopamine at the highest concentration (1000 μg/ml) caused distortion of the embryonic axis, general failure of somite and heart formation, and poor neural differentiation. Development of the embryo even to this extent was in sharp contrast to the growth of HeLa cells which is completely inhibited in the presence of dopamine (50 μg/ml) (10).

To estimate qualitatively the effect of dopamine on the hemoglobin formation, the whole blastoderm after incubation was stained with Ralph's benzidine stain (13), a very sensitive method for the detection of hemoglobin. Results showed (Fig. 1A-B) that

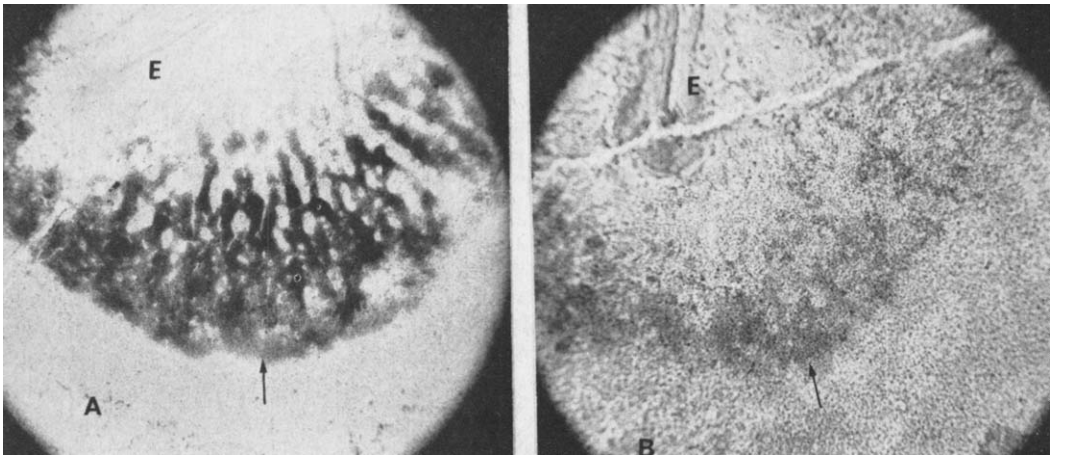


FIG. 1. Effect of dopamine (500 μg/ml) on the area vasculosa of chick blastoderm *in vitro*. After 24 hr of incubation the whole blastoderm was stained with Ralph's benzidine stain. Qualitative differences in intensity of hemoglobin stain in the area vasculosa (indicated by arrows) of control (A) and dopamine-treated blastoderm (B) are seen. "E" indicates the caudal portion of the embryo (×12).

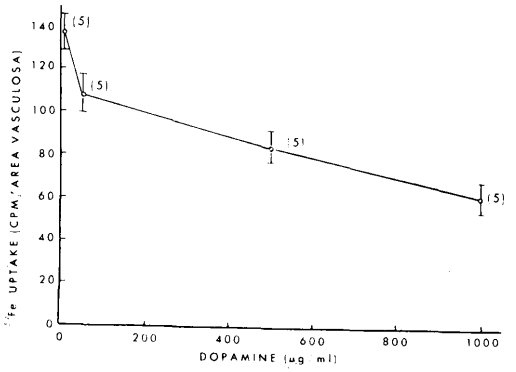


FIG. 2. Effect of dopamine on ^{59}Fe uptake in the area vasculosae of chick blastoderms. The number in parentheses represents the number of blastoderms. Vertical bars are SE of means (95% confidence interval).

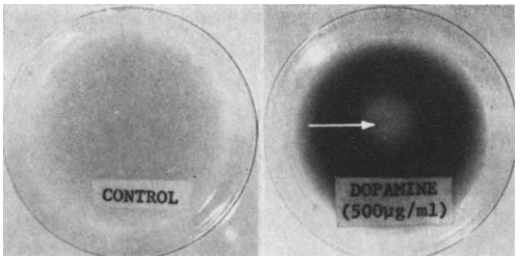


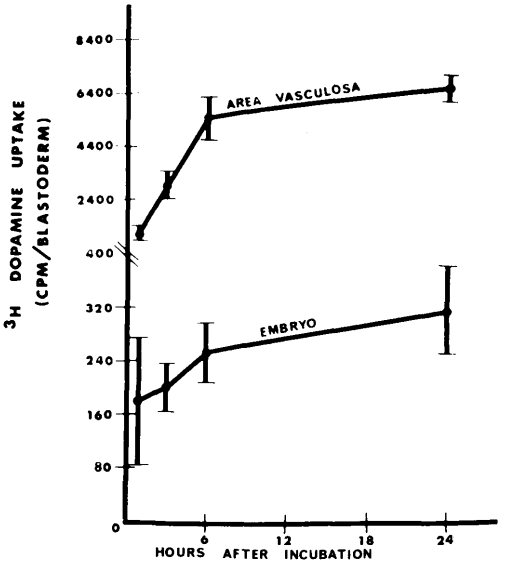
FIG. 3. A. Control and dopamine-treated culture medium after removal of the blastoderm at the termination of the experiment. The area (indicated by arrows) underneath the dopamine-treated blastoderm shows very little auto-oxidation. B. ^{3}H dopamine uptake in the embryos and area vasculosae of chick blastoderms *in vitro*. Each point represents an average of five blastoderms. Vertical bars are SE of means (95% confidence interval).

dopamine markedly reduced hemoglobin synthesis in the area vasculosae compared to the controls. This is further substantiated by the reduced ^{59}Fe uptake in the area vasculosae of dopamine-treated blastoderms (Fig. 2).

Paraffin sections of area vasculosa stained with hematoxylin–eosin showed that cellularity within the vascular channels of dopamine-treated blastoderms approximated the controls; however, this technique does not provide accurate estimate of cell number.

When blastoderms of stage 8 or 9 were used, the effect of dopamine on hemoglobin synthesis was less than that seen in the blastoderms of stage 6 or 7. This may indicate that cells which have already initiated the hemoglobin formation are not affected by dopamine.

Dopamine uptake. After 24 hr of incubation, the culture medium (500 and 1000 µg/ml) around the blastoderm turned dark, indicating the auto-oxidation of dopamine; in contrast, the medium beneath the blastoderm showed very little auto-oxidation (Fig. 3A). When the blastoderm was removed and the culture medium incubated for an additional 48 hr, the medium previously covered by the blastoderm showed no further oxidation, indicating that the blastoderm utilized the major portion of dopamine. The ^{3}H dopamine uptake by the blastoderm further substantiated this (Fig. 3B).



Effect of dopa, norepinephrine, epinephrine, and serotonin. L-dopa (50, 500, and 1000 µg/ml), a precursor of dopamine, produced changes similar to those in the dopamine-treated blastoderms; however, at a concentration of 500 µg/ml, suppression of hemoglobin formation and vascular development was less. This indicates that L-dopa may have an effect similar to that of dopamine and/or that L-dopa is converted to dopamine by the concomitant development of dopa-decarboxylase which begins to appear between 1–2 days of incubation (12). Unlike dopamine, norepinephrine and epinephrine at

a concentration of 500 μg/ml produced 100% mortality of the blastoderms. Serotonin at a similar concentration produced no visible effect on the developing blastoderms.

Discussion. The present data establish that dopamine markedly inhibits the formation of hemoglobin in 24-hr chick blastoderm *in vitro* without affecting the development of the embryo proper. Other studies have shown (14–18) a reduction of hemoglobin synthesis in the area vasculosa of developing chick blastoderms by methyl-norvaline, actinomycin D, proflavin, 8-azaguanine, and by inhibitors of protein synthesis. These studies indicate that hemoglobin formation in early chick blastoderm is sensitive both at the transcription and translation level. The importance of heme in the regulation of hemoglobin synthesis has been demonstrated by the fact that Δ-aminolevulinic acid, a precursor of heme prosthetic groupl induces the premature onset of hemoglobin formation at an earlier stage of development than the normal 6- to 7-somite embryo (18). The inhibition of hemoglobin synthesis by actinomydin D and 8-azaguanine is prevented in the presence of Δ-aminolevulinic (18). The mechanism of dopamine-induced suppression of hemoglobin formation is unknown. The fact that dopamine reduces hemoglobin synthesis in blastoderms of stage 6 or 7 more than in 8 or 9 suggests that cells which have already initiated the hemoglobin formation are not affected by dopamine.

Summary. Dopamine markedly inhibited the synthesis of hemoglobin in the area vasculosa of chick blastoderms *in vitro* without affecting the development of the embryos proper. This effect was more evident in young blastoderms than in old ones. L-dopa, a precursor of dopamine, suppressed hemoglobin formation to a lesser degree. Norepinephrine and epinephrine at a similar concentration were lethal to all blastoderms, whereas serotonin had no visible effect on the hemoglobin synthesis or the development of embryos.

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