Effect of Dihydroxyphenylethylamine (Dopamine) on Mammalian Cells *in Vivo* and *in Vitro*[1] (34229)

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The pharmacological effects of dopamine on non-nervous tissue have been known for some time (1–2); however, the role of this agent in the function of nervous tissue has been reported only recently (3–5). In addition, dopamine may be used as a protective agent against whole body X-irradiation. For example, when dopamine is given prior to whole body irradiation of rodents, it increases both the number of survivors and survival time (6–7). This protective effect is probably related in part to the fact that dopamine acts as a scavenger of free radicals (8). This agent also markedly reduces the synthesis of hemoglobin in cultured chick blastoderm without affecting the development of the embryo (9).

Because of these findings, we decided to measure the effects of dopamine on DNA and protein synthesis in mouse spleen. This organ seemed particularly desirable, since its splenic nerves are rich in dopamine (10), and the rate of dopamine metabolism in the spleen is slow (11). Since DNA synthesis was inhibited, it seemed likely that cell division was also delayed. To test this hypothesis, the effects of dopamine were studied on cultured HeLa cells.

Materials and Methods. Dopamine treatment. Dopamine HCl, obtained from Nutritional Biochemicals Inc. was dissolved in 0.05 *N* HCl (5 mg/ml). The acid pH of the dopamine solution was necessary to reduce auto-oxidation. Male albino mice (Swiss Webster, strain ICR) weighing 25–30 g were maintained under our laboratory conditions for 4–7 days before experimentation.

Incorporation of ^{3}H-thymidine. Animals were injected with ^{3}H-thymidine (sp act of 13 Ci/mmole; 1 μCi/g of body wt) ip at 0.5, 1, 3, 6, 12, 24, or 48 hr after the administration of dopamine (1 mg/mouse). Mice were decapitated in the cold room 1 hr later. The incorporation of ^{3}H-thymidine into newly synthesized DNA was investigated at 1 and 6 hr following the injection of different dopamine doses (0.01, 0.1, and 1.0 mg/mouse). Sham-treated animals received an equivalent volume of 0.05 *N* HCl, whereas controls were given no HCl before the administration of ^{3}H-thymidine.

DNA extraction and estimation. DNA from the spleen was extracted by Schmidt–Thannhauser's method (12) as modified by Munro (13). Minor changes were made to suit the present experimental condition as described below. Since spleen has a high DNase activity, a gentle homogenization is recommended (14). One-third of the spleen was excised, rinsed and homogenized in 2 ml of distilled water by passing the Teflon pestle through a Potter–Elvehjem homogenizer by hand 10 times. One ml of 0.6 *N* perchloric acid was added immediately and any remaining large clumps of fibrous tissues were removed from the precipitate. The precipitate was washed twice with 0.2 *N* $HClO_4$. The precipitate was hydrolyzed in 0.3 *N* OH at 37° for 2 hr, stirred with a wooden applicator thrice, and then cooled at room temperature. To assay radioactivity, 0.1 ml of the hydrolysate was transferred into a counting vial containing 10 ml of dioxane-solvent system. DNA and protein were reprecipitated with 1.2 *N* perchloric acid (final concentration 0.2 *N*), washed twice in 0.2 *N* $HC10_4$, hydrolyzed in 1 *N* $HClO_4$ at 70° for 40 min, stirred twice and centrifuged at 4°. One ml of DNA hydrolysate was diluted with 9 ml of

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water to make a 0.1 *N* perchloric solution. The amount of DNA was estimated by measuring the absorbance at 260 mμ using highly polymerized calf-thymus DNA as a standard. Protein contamination in the DNA fraction was corrected for by Lowry's method (15) using lyophilized human serum as a standard.

Incorporation of ^{14}*C-leucine.* Animals were injected with ^{14}C-leucine (sp act of 311 mCi/mmole; 0.2 μCi/g of body wt) at 3, 6, and 24 hr after administration of dopamine (1 mg/mouse). The animals were killed 1 hr later and the spleen was homogenized in distilled water. The incorporation of radioactive amino acid into TCA-insoluble protein was determined by a filter paper disk method (16). The protein was measured by the method of Lowry *et al.* (15).

Assay of radioactivity. The radioactivity in the DNA and TCA-insoluble protein fraction was assayed by a liquid scintillation β spectrometer. The quenching of the DNA samples was determined using ^{3}H-toluene as an internal standard and was consistently 3–5% of the total radioactivity; therefore, the data were not corrected for the quenching. Since the filter containing the protein precipitate was counted in a toluene system, no quenching was detected in assaying protein radioactivity. Results were calculated as cpm/mg of DNA or protein and then expressed as percentage of controls.

Radioautographs. To determine whether the decrease in the incorporation of ^{3}H-thymidine into DNA was due to a block in the G_1 phase (pre-DNA synthetic phase) or a net inhibition in DNA synthesis, the labeling index was estimated both in control and experimental animals following an injection of ^{3}H-thymidine. Four animals were injected intraperitoneally with ^{3}H-thymidine (10 μCi/mouse) 4 hr after the administration of dopamine (1 mg/mouse) and were killed 30 min later. Four controls were given an equivalent volume of 0.05 *N* HCl. The procedure for the radioautographic technique is described elsewhere (17). A total of 2000–2500 cells were counted. Cells with nuclear grains of three and above were scored, and the number of labeled cells was expressed as percentage of total cells.

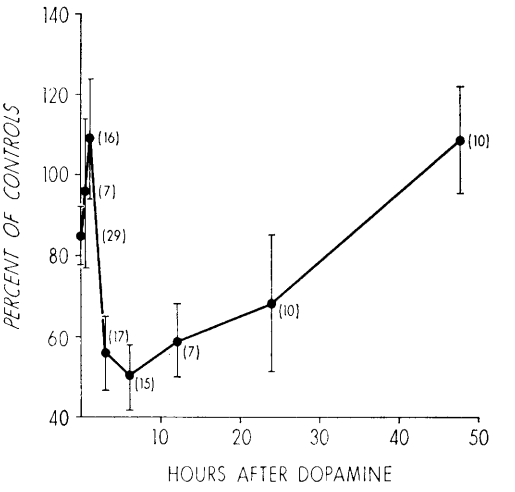

FIG. 1. Effect of dopamine (1 mg/mouse) on the incorporation of ^{3}H-thymidine into DNA of mouse spleen as function of time after the administration of dopamine. The average value of 18 controls receiving no treatment before the injection of ^{3}H-thymidine was used as 100% and has a SE of ± 18%. Sham-treated animals receiving an equivalent volume of 0.05 *N* HCl were used as the zero time group. The number of animals is given in parenthesis; vertical bars represent SE.

Studies on HeLa cells. In order to extend the *in vivo* findings, the effects of dopamine were studied on cultured HeLa Chessen cells. The procedure for cell maintenance, measurements of cell proliferation, labeling with ^{3}H-thymidine, and radioautographic procedures have been previously described by Kollmorgen *et al.* (18). Several concentrations of dopamine (5, 25, and 50 μg/ml) were added to the culture medium and the increase in cell number was determined as a function of time after the addition of this amine. The radioautographic preparation of cells labeled with ^{3}H-thymidine was made as a function of time after the addition of dopamine. The labeling index following ^{3}H-thymidine and the number of grains per cell were determined.

Results. Effect of dopamine on DNA synthesis and percentage labeling index of ^{3}*H-thymidine.* Figure 1 shows the incorporation of ^{3}H-thymidine into spleen DNA as a function of time after the administration of dopamine. The incorporation value of ^{3}H-thymidine in sham-treated animals receiving

an equivalent volume of 0.05 *N* HCl was used as the zero time and was not significantly different from that of controls receiving no treatment. In dopamine-treated mice, the incorporation of ^{3}H-thymidine into spleen DNA was reduced to 50% of controls at 3 and 6 hr after the administration of the amine. This effect was reversible within 48 hr. The incorporation of ^{3}H-thymidine into DNA as a function of dopamine concentration (0.01, 0.1, and 1 mg/mouse) at 1 and 6 hr after injection was measured. At 6 hr dopamine at a dose of 0.1 mg and 1 mg/mouse reduced the incorporation of ^{3}H-thymidine into DNA by 60± 12 and 50 ± 8%, respectively, whereas at 1 hr dopamine produced no significant change in isotopic content. Dopamine at a dose of 0.01 mg/mouse did not affect spleen DNA synthesis. The percentage of labeled cells in dopamine-treated animals was 10.2, not significantly different from sham-treated controls.

Effect of dopamine on protein synthesis. The incorporation of ^{14}C-leucine into TCA insoluble protein was not significantly affected by dopamine.

Effect of dopamine on HeLa cells. Figure 2 shows that the growth of HeLa Chessen cells was altered with concentrations as low at 5 μg/ml of medium. Increasing the concentration fivefold altered the growth process more

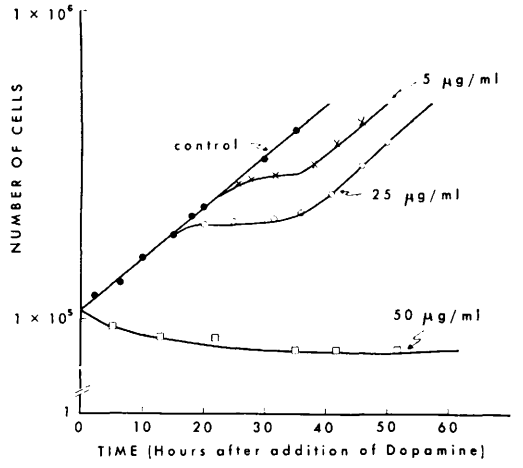

FIG. 2. Number of cells is plotted as a function of time. The concentration given is for dopamine in the medium. The standard error of each point is approximately equal to the size of the symbol.

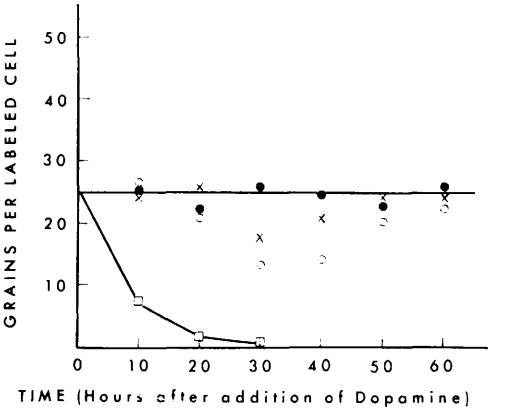

FIG. 3. Number of grains per labeled cells after a 15-min pulse with ^{3}H-thymidine is plotted as a function of time: (●), control cells; dopamine in media (μg/ml): (×), 5; (○), 25; (□) 50. The standard error of each point is approximately equal to the size of the symbol.

substantially and more quickly. However, after this transient effect, surviving cells retained the 18-hr doubling time of control cells. When doses of 50 μg/ml or greater were used, cell division was inhibited immediately, and within 3 days all cells were dead. The percentage of cells synthesizing DNA remain unchanged at about 37% measured at 10-hr intervals up to 60 hr after the addition of dopamine (5 and 25 μg/ml). However, at a concentration of 50 μg/ml, the percentage of labeled cells was reduced to about 15% at 10 hr and to less than 1% at 40 hr after the addition of dopamine. The SE for these values was about ±2. Figure 3 shows that dopamine inhibited the incorporation of ^{3}H-thymidine per cell and the extent of depression appears to be dose-dependent. However, the DNA synthetic phase is not the only phase of the generation cycle affected by dopamine. The duration of G_1, G_2, and M is also increased proportionately.

Discussion. Dopamine is essential for the function of nervous tissue, both central (3–5) and peripheral (10, 19, 20). Our previous studies (6–9) have shown that dopamine also affects the function of non-nervous tissue. The present paper demonstrates that dopamine inhibits spleen DNA synthesis without affecting protein synthesis. The mechanism of dopamine-induced inhibition of DNA syn-

thesis is unknown; however, this is not caused by a "blockade" of cells in any phase of the cell cycle because the labeling index in spleen cells of dopamine-treated animals is similar to that of sham-treated animals. Some recent data from our laboratory indicates that dopamine interferes with iron metabolism. The uptake of ^{59}ferrous sulfate is also significantly reduced in cultured chick blastoderm when dopamine is present (9).

The *in vivo* findings have been substantiated by the *in vitro* study. Dopamine also inhibits the incorporation of ^{3}H-thymidine into newly synthesized DNA *in vitro,* whereas the percentage of cells synthesizing DNA remains unchanged. In addition to this, the extent of depression of DNA synthesis in HeLa Chessen cells appears to be dose-dependent. The fact that the duration of G_1, S, G_2 and M is not differentially altered, and the observation that the distribution of the population in the various phases of the cell cycle does not change, indicates that the effects of dopamine are fairly uniform and not confined to any specific phase of the cell cycle. Dopamine at concentrations of 50 μg/ml and above completely inhibits the growth of HeLa Chessen cells. These cells are much more sensitive to this agent than cultured chick blastoderm *in vitro* which shows no effect in the presence of 200 μg/ml of dopamine (9).

The oxidation products of dopamine are toxic to cultured cells. Since dopamine is utilized by the cultured cells, we presume that at a low amine concentration, oxidation products are not formed in a significant amount during our period of observation. Since the rate of auto-oxidation is time-dependent, oxidation products of dopamine are not expected to show an immediate effect even at a higher concentration.

It appears that non-nervous cells might be more sensitive to dopamine than the nerve cells. This is substantiated by our earlier work in which the hemoglobin formation of chick blastoderm *in vitro* shows greater sensitivity than the neural development. This may be related to the fact that the dopamine pathway is present only in nervous tissue. The present evidence along with earlier reports (6–9) suggests that dopamine not only constitutes an important neurohormone, but it also affects the function of non-nervous tissue.

Summary. Dopamine (0.1 and 1 mg/mouse) inhibited DNA synthesis in mouse spleen. This effect was reversible and was not related to the availability of fewer cells in S phase because the labeling index of spleen cells was similar in both dopamine-treated and control animals. Dopamine produced no change in the rate of synthesis of spleen protein.

Dopamine (50 μg/ml) completely inhibited the growth of HeLa Chessen cell populations *in vitro*. Dopamine at a low dose (5 to 25 μg/ml) produced only a partial inhibition of the growth. Both the onset of division delay and the time required for recovery are dose-dependent in this dose range. Dopamine also reduced the incorporation of ^{3}H-thymidine into newly synthesized DNA, whereas the percentage of cells synthesizing DNA remained unchanged.

1. Barger, G. and Dale, H. H., J. Physiol. **41**, 19 (1910).

2. Holtz, P., Heise, R., and Ludtke, K., Arch. Exptl. Pathol. Pharmakol. **191**, 87 (1939).

3. Blaschko, H., Experientia **13**, 9 (1957).

4. Carlsson, A., Lindqvist, M., and Magnusson, T., Nature **180**, 1200 (1957).

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

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

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

8. Prasad, K. N. and VanWoert, M. H., Intern. J. Radiation Biol. **14**, 79 (1968).

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

10. Von Euler, U. S. and Lishajko, F., Acta Physiol. Pharmacol. Neerl. **6**, 295 (1957).

11. Symchowicz, S. and Korduba, D. A., Biochem. Pharmacol. **16**, 385 (1967).

12. Schmidt, G. and Thannhauser, S. J., J. Biol. Chem. **161**, 83 (1945).

13. Munro, H. N., Methods Biochem. Analy. **14**, 113 (1966).

14. Bowers, W. E., Finkenstaedt, J. T., and DeDuve, C., J. Cell Biol. **32**, 325 (1966).

15. Lowry, O. H., Rosebrough, N. J., Farr, A. L.,

and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

16. Mans, R. J., and Novelli, G. D., Arch. Biochem. Biophys. **94**, 48 (1961).

17. Prasad, K. N., J. Exptl. Zool. **166**, 355 (1967).

18. Kollmorgen, G. M. and Griffin, M. J., Cell Tissue Kinet. **2**, 111 (1969).

19. Laverty, R. and Sharman, D. F., Brit. J. Pharmacol. **24**, 538 (1965).

20. Schumann, H. J., Arch. Exp. Path. Pharmak. **227**, 566 (1956).

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