Effect of Dopamine and 6-Hydroxydopamine on Mouse Neuroblastoma Cells in Vitro

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SUMMARY

Dopamine and to a much greater extent, 6-hydroxydopamine reduced the growth of the mouse neuroblastoma cell population in vitro but did not induce morphological differentiation. The growth inhibition at a lower drug concentration was primarily due to inhibition of cell multiplication, which was reversible. L-Dopa, norepinephrine, epinephrine, 3,4-dihydroxyphenylacetic acid, or homovanillic acid at a similar concentration did not affect the growth of the neuroblastoma cell population in vitro. Dopamine and 6-hydroxydopamine have little or no effect on the growth of Chinese hamster ovary (CHO-K1) and baby hamster kidney (BHK-21) cell populations in vitro.

INTRODUCTION

The importance of dopamine in the central and peripheral nervous tissue has been established (3); however, the significance of dopamine for the proliferating nerve cells has not been investigated. The availability of mouse NB in vitro provides an opportunity to study the above problem. Several neuronal features of mouse NB cells in vivo and in vitro have been established (2, 5—12).

6-Hydroxydopamine produces a selective destruction of sympathetic cells in newborn and young animals (1, 13). Since the NB cells are of sympathetic origin, the effect of 6-hydroxydopamine on the growth of NB cell populations in vitro is investigated.

This paper shows that dopamine and 6-hydroxydopamine markedly inhibited the growth of the mouse NB cell population in vitro. Norepinephrine, epinephrine, L-dopa, or acid metabolites of dopamine had no effect on the growth. Dopamine and 6-hydroxydopamine at a comparable concentration did not affect the growth of nonnerve cells.

MATERIALS AND METHODS

Chemical and Radioisotopes. Dopamine (3,4)-dihydroxyphenylethylamine-HCl, Nutritional Biochemicals Corp., Cleveland, Ohio), L-dopa (L-dihydroxyphenylalanine, Nutritional Biochemicals Corp.), DL-norepinephrine, L-epinephrine (K & K Laboratories, Plainview, N. Y.) and homovanillic acid (Regis Chemical Co., Chicago, Ill.) were obtained from the commercial sources. Dopamine-3H (side chain 1,2,3H; specific activity, 2.2 Ci/mM), norepinephrine-3H (DL-norepinephrine-7-3H; specific activity, 0.25 µCi/0.0048 mg), and epinephrine-3H (DL-epinephrine-7-3H; specific activity, 0.25 µCi/0.00043 mg) were obtained from Amersham/Searle Corp., Arlington Heights, III.

Cell Culture. In our laboratory, mouse NB cells have been in culture for more than 8 months. Cells were grown as a monolayer in Falcon plastic flasks containing F12 medium with 10% gamma globulin newborn calf serum, penicillin (100 µg/ml), and streptomycin (100 units/ml) and were maintained at 36°C in a humidified atmosphere of 5% CO2 in air. Cells have not been cloned. When grown in Falcon plastic flasks, the average doubling of time of NB cells is 18 to 20 hr, which is consistent with the value published by others (4, 7). Our NB cell line has acetylcholinesterase but no butyrylcholinesterase (8). Chinese hamster ovary (CHO-K1) and baby hamster kidney (BHK-21) cells are grown under experimental conditions identical with those of NB cells and have doubling times of 12 to 13 and 8 hr respectively.

Treatment with Drugs. Cells were treated with one of the drugs 24 hr after plating (10^5 cells). Dopamine was dissolved in F12 medium without serum (neutral pH) immediately before experimentation and added to NB cells in vitro at a concentration of 10 or 50 µg/ml. After 24 hr of incubation, the medium turned dark, indicating the autooxidation of dopamine. Only a few cells remained in the flask 72 hr after treatment, suggesting that autooxidation products of dopamine were toxic. For minimization of the toxic effect of autooxidation products, dopamine was dissolved in the F12 medium without serum containing ascorbic acid, 1 mg/ml, and added to the NB cell culture to give final concentrations of 10, 50, 100, and 200 µg of dopamine per ml and of 20 µg of ascorbic acid per ml. After 1 hr of incubation, the cells were washed twice, and fresh growth medium was added. Ascorbic acid under the above experimental conditions did not affect the growth or survival of NB cells in vitro. CHO-K1 and BHK-21 cells were treated with dopamine in the manner described above.

For investigation of the specificity of dopamine action, the effect of norepinephrine, epinephrine, dopa, 3,4-dihydroxyphenylacetic acid, and homovanillic acid on the growth of NB cell populations in vitro was investigated.

Received February 2, 1971; accepted May 21, 1971.
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DL-Norepinephrine, 3,4-dihydroxyphenylacetic acid, or homovanillic acid was dissolved in the F12 medium without serum immediately before use. Since L-epinephrine was insoluble in the medium, it was first dissolved in 1 ml of 0.01 N HCl; then 3 ml of F12 medium without serum were added to give a final concentration of 5 mg/ml. L-Dopa was also insoluble in the medium and therefore was dissolved in 0.1 N HCl in F12 medium without serum. NB cells were treated with one of the drugs at the same concentration and under the same experimental conditions as those described for dopamine. Control cells were treated similarly, except that no drug was added.

6-Hydroxydopamine is also rapidly autooxidized in solution. Therefore it was dissolved in F12 medium without serum containing ascorbic acid, 1 mg/ml, immediately before use. NB, CHO-K1, and BHK-21 cells were incubated in the presence of 6-hydroxydopamine (1, 5, 10, and 20 µg/ml and ascorbic acid (20 µg/ml) in the manner described for dopamine.

Measurement of Survival, Growth, and Differentiation. The growth of the cell population was determined by counting with a hemocytometer or Coulter counter the total cell number as a function of time after treatment. For single cell suspensions, cells were incubated in 0.25% Viokase solution for 20 min. The cells stained with trypan blue (0.1% in 0.9% NaCl solution) were considered dead and were subtracted from total counts. Since the percentage of dead cells in control and drug-treated populations was always less than 1, the counting in the subsequent experiments was done in a Coulter counter.

The cell number in control and drug-treated cell populations was counted, and growth inhibition 1, 2, and 3 days after drug treatment was calculated as follows:

\[
\left[ \frac{\text{No. of control cells} - \text{no. of drug-treated cells}}{\text{no. of control cells}} \right] \times 100
\]

Cytoplasmic extensions greater than 50 μm long were considered as axons, and the formation of axons was regarded as an expression of morphological differentiation.

Uptake of Dopamine-3H, Norepinephrine-3H, and Epinephrine-3H. Dopamine-3H was diluted with F12 medium containing ascorbic acid, 1 mg/ml, whereas norepinephrine-3H and epinephrine-3H were diluted with 0.9% NaCl solution. NB cells (3 days after plating) were incubated in the presence of 1 of the radioactive amines (0.5 µCi/ml) for 1 hr, washed twice with 0.9% NaCl solution, and removed from the flask by incubating the cells in 0.25% Viokase solution for 20 min. An aliquot (0.2 ml) was taken for counting, and the remainder was centrifuged. The pellet was digested in 0.5 ml of solubilizer (NCS) overnight. The radioactivity was assayed in a liquid scintillation β-spectrometer with a toluene solvent system (4 g of PPO per liter of toluene). The uptake of dopamine-3H by BHK-21 and CHO-K1 cells was studied in a similar fashion.

Data were expressed as cpm/10^6 cells.

RESULTS

Mouse NB cells in vivo represented a homogenous cell population consisting of small round cells and a few large polyploids; however, in monolayer culture a few big cells morphologically resembled neurons with large nuclei and 1 or more axons. Although the percentage of large cells in the single cell suspension varied from 4 to 8, only a few neurons were seen when cells were stained in the attached condition, indicating that most of the large cells were polyploids. Most of the small cells grew on top of each other and formed clumps, whereas others sent out small cellular processes. These processes were not considered axons.

Effect of Dopamine on NB, CHO-K1, and BHK-21 Cells. Dopamine inhibited the growth of the NB cell population in vitro, and this effect was dose dependent (Chart 1); however, no morphological differentiation was introduced. The cell number 1 day after dopamine treatment (100 µg/ml) did not change, and the viability of drug-treated cells was similar to that of controls. At a higher dopamine concentration (200 µg/ml), the cell number decreased, indicating that in addition to inhibition of cell division cell death occurs at this dose. The inhibition of cell division was reversible, and drug-treated cells during the 2nd and 3rd days of treatment grew with the same doubling time as that of controls. This is substantiated by the fact that the percentage of growth inhibition 2 and 3 days after drug treatment was similar. In dopamine-treated cell populations, the tendency of cells to pile on top of each other was similar to the controls. The growth of CHO-K1 was less sensitive to dopamine than were NB cells; however, the growth of BHK-21 cells was not affected at all (Chart 1).

Effect of Other Catecholamines and a Precursor and Metabolites of Dopamine. For investigation of the specificity of dopamine-induced inhibition, the effect of norepinephrine, epinephrine, dopa, 3,4-dihydroxyphenylacetic acid, and

![Chart 1. Percentage of inhibition as a function of dopamine concentration. The percentage of inhibition 2 days after treatment was calculated as follows: [(no. of control cells - no. of drug-treated cells)/no. of control cells] x 100. Each value represented an average of 6 to 8 samples. Vertical bars, S.D. The standard deviations of points not shown in the chart were approximately equal to the size of the symbols.](chart1.png)
Dopamine, 6-Hydroxydopamine, and NB Cells

Table 1

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Dopamine-3H</th>
<th>Norepinephrine-3H</th>
<th>Epinephrine-3H</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB</td>
<td>1336 ± 356°</td>
<td>906 ± 196</td>
<td>429 ± 172</td>
</tr>
<tr>
<td>CHO-K1</td>
<td>166 ± 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHK-21</td>
<td>747 ± 200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a S.D.

Chart 2. The percentage of inhibition as a function of 6-hydroxydopamine concentration was calculated 2 days after treatment. Each value represents an average of 5 to 6 samples. Vertical bars, S.D. The standard deviations of points not shown in the figure were approximately equal to the size of the symbols.

Dopamine and, to a much greater extent, 6-hydroxydopamine markedly inhibit the growth of mouse NB cell populations in vitro. The dopamine effect appears to be specific, because neither other catecholamines (norepinephrine and epinephrine), L-dopa, nor acid metabolites of dopamine at a similar concentration have any effect on the growth of NB cells in vitro. Dopamine- or 6-hydroxydopamine-induced growth inhibition of NB cells is primarily due to inhibition of cell division. This is demonstrated by the fact that the cell number 1 day after treatment does not change significantly and the viability of the drug-treated cell population is similar to that of controls. At a higher drug concentration, cell death also contributes to the growth inhibition. The inhibition of cell multiplication is reversible, and cells grow with the same doubling time as that of controls. This is substantiated by the fact that the percentages of growth inhibition 2 and 3 days after drug treatment are similar. This compound at a comparable concentration did not inhibit the growth of CHO-K1 or BHK-21 cells in vitro (Chart 2).

DISCUSSION

Dopamine and, to a much greater extent, 6-hydroxydopamine markedly inhibit the growth of mouse NB cell populations in vitro. The dopamine effect appears to be specific, because neither other catecholamines (norepinephrine and epinephrine), L-dopa, nor acid metabolites of dopamine at a similar concentration have any effect on the growth of NB cells in vitro. Dopamine- or 6-hydroxydopamine-induced growth inhibition of NB cells is primarily due to inhibition of cell division. This is demonstrated by the fact that the cell number 1 day after treatment does not change significantly and the viability of the drug-treated cell population is similar to that of controls. At a higher drug concentration, cell death also contributes to the growth inhibition. The inhibition of cell multiplication is reversible, and cells grow with the same doubling time as that of controls during the 2nd and 3rd days of treatment. This is substantiated by the fact that the percentages of growth inhibition 2 and 3 days after drug treatment are similar. This study shows that mouse NB cells are more sensitive to dopamine and 6-hydroxydopamine than the nonnerve cells. The mechanism of these effects is known. It remains to be seen whether such a preferential effect of dopamine and 6-hydroxydopamine on NB cells in vitro can be demonstrated.

We have shown recently that X-irradiation (6) or dibutyryl adenosine 3':5'-cyclic monophosphate treatment (7) of mouse NB cells in vitro induces morphological differentiation as evidenced by the formation of axons. These cells appear to undergo morphological maturation as shown by the enlargement of the cells and their nuclei and the appearance of granular cytoplasm. Like X-rays, dopamine and 6-hydroxydopamine also inhibit cell division in vitro, but these compounds do not induce morphological differentiation. Therefore the inhibition of cell division is necessary but not mandatory for morphological cell differentiation.

REFERENCES

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*Cancer Res* 1971;31:1457-1460.

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