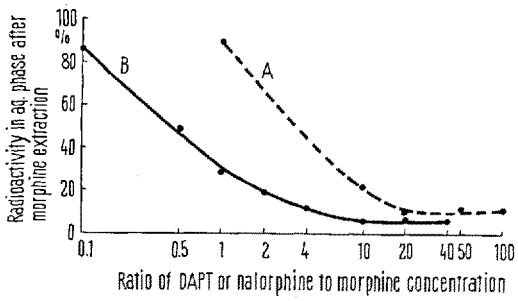

Fig. 3. Effect of different concentrations of 2,4-diamino-5-phenyl thiazole hydrobromide DAPT (A) and nalorphine (B) on conversion of morphine-N-methyl-C^{14} to water-soluble products by peroxidase.

Zusammenfassung. In Modellsystemen von Peroxidase, Proteinen und Metallionen wird Morphium in wasserlösliche (Radikal-)Produkte übergeführt, welche Bindungen mit dem Protein eingehen.

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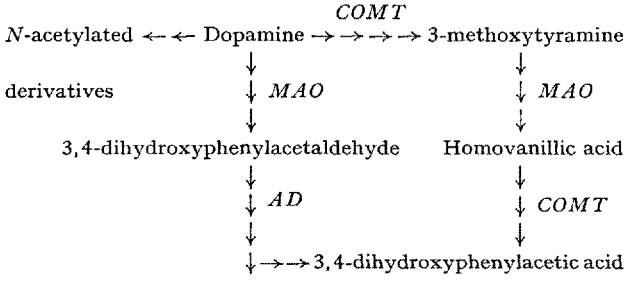
Iproniazid (Marsilid) Reverses Dopamine-Effect and Stimulates the Growth of Mouse Neuroblastoma in vitro

Dopamine markedly inhibits the cell division of neuroblastoma (NB) cell in vitro. On removal of drug 1 h after treatment, the inhibition of cell multiplication persists for 24 h after which dopamine-treated cells grow with same doubling time as that of controls. The precursor of dopamine (L-dopa), metabolite of dopamine (homovanillic acid), norepinephrine or epinephrine produces no such effect[1]. Dopamine at a similar concentration does not affect the cell division of Chinese Hamster-ovary-K1 and Baby-hamster kidney-21 cells in vitro[1]. To elucidate the mechanism of dopamine-effect further, the inhibitor of dopamine metabolism is used in this investigation. The metabolic pathway of dopamine in mammalian cells is shown diagramatically below:



Dopamine is deaminated by monoamine oxidase (MAO) to form aldehyde which is rapidly oxidized by aldehyde dehydrogenase (AD) to produce 3,4-dihydro-xyphenylacetic acid which is converted to homovanillic acid by catechol-*O*-methyl-transferase (COMT). The other metabolic pathway is self explained in the diagramatic presentation. This paper shows that iproniazid (marsilid), an inhibitor of monoamine oxidase, completely reverses dopamine-effect on neuroblastoma cells and by itself stimulates the cell division of neuroblastoma as well as of Baby-hamster kidney cells in vitro. Pyrogyllol, an inhibitor of COMT, markedly reduces the growth of neuroblastoma cells.

Materials and methods. The procedure for culturing and the morphological features of neuroblastoma cell line has been described in the previous publication[1]. When grown in Falcon plastic flask, the average doubling time of neuroblastoma cells is 24 h. This cell line has acetylcholinesterase activity, but no butyrylcholinesterase, indicating its neuronal feature[2]. In addition, tyrosine hydroxylase, a rate limiting enzyme in the biosynthesis of catecholamine is present in the primary tumor[3,4], but is lost in the long-term culture; however the enzyme activity is restored in the dibutyryl cyclic AMP-induced differentiated neuroblastoma cells (PRASAD, WAYMIRE and WEINER, in preparation).

Neuroblastoma cells were treated with marsilid or pyrogyllol 24 h after plating. Marsilid was dissolved in F12 medium without serum immediately before the experiment and added to neuroblastoma cells in vitro at a concentration of 100 µg/ml. Dopamine, 3'4' dihydroxyphenylacetiacid (DPAA) or pyrogyllol was dissolved in the F12 medium without serum containing 1 mg/ml of ascorbic acid immediately before the experiment. Dopamine was added to neuroblastoma cell culture 18 h after the addition of marsilid or 1 h after the addition of pyrogyllol to give a final concentration of dopamine (50 and 100 µg/ml) and of ascorbic acid (20 µg/ml). DPAA was added to neuroblastoma cells (50 and 100 µg/ml) 24 h after plating. After 1 h of incubation, cells were washed twice with F12 without serum and fresh growth medium was added. Control cell population was treated similarly except no drug was added. The addition of ascorbic acid was necessary to prevent the auto-oxidation of dopamine in vitro. Ascorbic acid by itself had no effect on neuroblastoma cells. The cell number 2 days after dopamine-treatment was counted by a Coulter counter. The growth inhibition of neuroblastoma cell population in vitro was calculated as follows:

$$\frac{\text{No. of control cells} - \text{No. of drug-treated cells}}{\text{No. of control cells}} \times 100.$$

If the number of drug-treated cells was greater than the control, the value obtained by the above formula was considered as an index of growth stimulation.

BHK-21 cells in vitro were grown under conditions identical to those of neuroblastoma cells. Marsilid (100 µg/ml) was added to BHK-21 cell culture 24 h after plating (50,000 cells). After 18–20 h of incubation, cells were

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Effect of marsilid on dopamine(DA)-effect

Treatment	Cell type	Growth of cell population Inhibition (%)	Stimulation (%)
DA (50 µg/ml)	NB	56.0 ± 6.0	
DA (50 µg/ml) + marsilid	NB	—	10.0 ± 8.0[a]
DA (100 µg/ml)	NB	71.0 ± 4.0	—
DA (100 µg/ml) + marsilid	NB	2.0 ± 5.0	—
Marsilid	NB	—	21.0 ± 4.0
Marsilid	BHK-21	—	27.0 ± 4.0

[a] Standard deviations. Marsilid (100 µg/ml) was added 24 h after plating. After 18–20 h of incubation in the presence of marsilid, dopamine (50 and 100 µg/ml) was added and then incubated for 1 h. After incubation cells were washed twice with F12 medium without serum and fresh growth medium was added. The total number of cells was counted 2 days after dopamine-treatment. The experiment was repeated thrice and each value represented an average of at least 6 samples.

washed twice and fresh growth medium was added. The cell number in drug-treated and control neuroblastoma culture was compared 3 days later.

Results and discussion. The Table shows that marsilid completely prevented the dopamine-induced inhibition of cell division. Dihydroxyphenylacetic acid (DPAA) an acid metabolites of dopamine did not effect the growth of neuroblastoma cells. MAO converts 3,-methoxytyramine, an intermediate metabolite of dopamine, to homovanillic acid; however, homovanillic in the previous study[1] produced no effect on the growth. These results indicate that amine-dervived aldehyde may be responsible for the dopamine-effect. This study also shows that marsilid stimulated the growth of neuroblastoma as well as of Baby-hamster kidney cells in vitro (Table), indicating that this marsilid-effect is not specific for neuroblastoma cells. The growth medium in the presence of pyrogyllol (100 µg/ml) turned dark-brown within 1 h after treatment, indicating the auto-oxidation of the compound. Addition of ascorbic acid (20 µg/ml) did not prevent the auto-oxidation. The COMT inhibitor under above experimental condition reduced the growth of neuroblastoma cells to about 6% of controls. The mechanism of marsilid-induced stimulation of cell division is unknown. However, it may be postulated that cellular-aldehyde may be one of the important factors in controlling the cell division.

Zusammenfassung. Die hemmende Wirkung von Dopamin auf die Zellteilung von Neuroblastoma beruht wahrscheinlich nicht auf dem Effekt seines desaminierten Metaboliten, obwohl sie durch den MAO-Hemmer Marsilid aufgehoben wird.

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[5] Acknowledgment. This investigation was supported by PHS Research Grant No. NS90230 from the National Institute of Neurological Disease and Stroke and by Grant No. DRG 1041 from the Daymon Runyon Memorial Fund for Cancer Research. I thank Mrs. ARPIL MONTGOMERY and MARIANNE GASHLER for technical help.

Effect of Catatoxic Steroids upon Established Morbid Changes

A considerable amount of evidence has accumulated during recent years in support of the concept that catatoxic steroids exert most of their protective effects by destroying toxicants (e.g., through the induction of hepatic microsomal or other enzymes). Thus, pretreatment with spironolactone, ethylestrenol, norbolethone and many steroid carbonitriles has been shown to offer protection against the subsequent administration of digitoxin[1], mercury[2], pesticides[3], carcinogens[4], nicotine[5], excessive amounts of steroid hormones[6,7], tyrosine[8], and well over 100 other toxicants, widely differing in their chemical structure and pharmacologic actions[9]. These findings suggest interesting clinical applications in the prevention of diseases caused by exogenous or endogenous intoxications.

Defensive enzyme induction by these steroids may be more or less rapid but usually several days of pretreatment are necessary to obtain optimal protection. Some catatoxic effects do develop more rapidly, but of course immediate improvement of intoxications cannot be expected from substances which act through the time-taking process of defensive enzyme synthesis and do not neutralize toxicants directly, nor block their damaging actions at the level of the target organs.

In view of these considerations, the clinical applicability of catatoxic steroids appeared to be limited to the prophylaxis of predictable intoxications, such as unavoidable transient occupational exposure to pesticides, carcinogens, mercurials or other chemicals amenable to this type of detoxication. It was thought that by the time spontaneous diseases (caused by endogenous toxicants or accidental poisoning with exogenous materials) would produce symptoms permitting a diagnosis, the catatoxic steroids could no longer be of avail since they do not affect the established lesions themselves.

To check this assumption, 2 experiments were performed: the first to determine the duration of catatoxic steroid pretreatment required to obtain protection; the second to verify whether an already manifest intoxication could be cured by merely blocking the continued pathogenic action of the causative agent.

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