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MOUSE NEUROBLASTOMA CLONE N1E-115: A SUITABLE MODEL FOR STUDYING THE ACTION OF DOPAMINE AGONISTS ON TYROSINE HYDROXYLASE ACTIVITY*

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Abstract—The DOPA-content in neuroblastoma clone N1E-115 is higher than the dopamine or noradrenaline content. Blockade of tyrosine hydroxylase by α-methyl-*p*-tyrosine (1×10^{-3} M) resulted in a decrease of cellular DOPA-content to 24.9% after 4 hr. The accumulation of DOPA in these cells which is probably due to limited activity of *l*-aromatic amino acid decarboxylase led us to use DOPA-content as a measure of tyrosine hydroxylase (TH) activity. Dopamine and especially apomorphine were effective at low concentrations (dopamine IC_{50} 1×10^{-5} M, apomorphine 2×10^{-7} M); lisuride had no effect on TH-activity. The low effective dose of apomorphine and the failure of lisuride to influence TH-activity are comparable to the observations in striatal synaptosomal preparations and make the N1E-115 clone a suitable model for studying the mechanism of TH-regulation. However, since haloperidol (1×10^{-5} M) did not reverse the apomorphine-induced blockade of TH, a receptor-mediated blockade of TH seems to be improbable.

After administration of the antimetabolite 6-aminonicotinamide to rats we found characteristic symptoms of disturbed neurological function which were interpreted as a Parkinson-like syndrome [1]. The most conspicuous phenomena were the decrease in dopamine concentration in the striatum and the occurrence of a pathologically enhanced muscular rigidity which could be normalized by administration of L-DOPA and of dopamine agonists [2].

These findings induced us to study the regulation of the catecholamine synthesis by dopamine agonists on genetically defined clonal nerve cell lines. It is known that murine neuroblastoma cells in culture possess a variety of properties of normal neurons, and that several clonal lines have the capacity to both synthesise and degrade catecholamines [3], but the yield in catecholamines is, in general, very low. It was important to find a clonal nerve cell line with a high tyrosine hydroxylase (TH) activity, since TH is usually considered as the rate-limiting enzyme in catecholamine synthesis.

The clone N1E-115, in which very high levels of TH-activity as well as high levels of dopamine-β-hydroxylase (DβH) activity were found, seemed to be particularly suitable for such investigations. In contrast, the activity of the *l*-aromatic amino acid decarboxylase (AAAD) was very low which corresponds with observations made on other clones of similar origin [4]. However, several other authors reported at least some level of dopamine and noradrenaline production [5, 6]. Preliminary studies in our laboratories showed that N1E-115 cells indeed contain a clearly higher level of DOPA than of dopamine or noradrenaline.

In animal experiments DOPA-levels after pharmacological blockade of AAAD are used as *in vivo* measure to assess TH-activity [7]. Starting from a similar hypothesis, we report in this paper that drug effects on TH-activity are reflected by changes in the DOPA-content of N1E-115 cells. We believe that this model can be used to elucidate the regulation of TH-activity in intact neural cells.

MATERIALS AND METHODS

Cells and culture conditions. Mouse neuroblastoma clone N1E-115 (a gift of Prof. U. Littauer, Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot/Israel) were cultured on monolayer in Dulbecco's modification of Eagle's medium containing 1 mM L-glutamine, 500 U/ml penicillin, 500 μg/ml streptomycin, and 2.5 ml/l non-essential amino acid (NEAA) concentrate. The medium was supplemented with 10% v/v fetal calf serum (all obtained from Seromed, München, F.R.G.). Cells were grown in flasks (75 cm^2/250 ml, Lux Scientific Corporation, Newbury Park, CA) in an atmosphere of 7% CO_2 and 93% humidified air at 37° with 20 ml of medium.

On the 7th day after plating (cells had reached the confluent stage) the medium was replaced. One hour later the drug or the appropriate control solution was added to the medium, total incubation time with the drug was 4 hr.

Catecholamine assay. At the end of the incubation period the flasks were rinsed three times with ice-cold Hanks' balanced salt solution (Seromed, München, F.R.G.) and then 4 ml 0.4 M PCA containing 0.25% EDTA and 0.06% $Na_2S_2O_5$ were added. The cells of two flasks were scraped off, pooled, sonicated and centrifuged at 30,000 *g* for 10 min. The extract was purified and concentrated on a strong cation exchange column (Dowex 50 W X-

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Table 1. Tyrosine- and catecholamine-content related to mg protein (mean ± S.E.M.) in N1E-115 clone on the seventh day after plating (cells had reached the confluent stage)*

	Tyrosine (μg/mg protein)	DOPA (ng/mg protein)	Dopamine (ng/mg protein)	Noradrenaline (ng/mg protein)
Fluorimetric determination	1.3 ± 0.1 (n = 13)	15.7 ± 1.3 (n = 46)		7.5 ± 0.7 (n = 47)
HPLC with electrochemical detection		10.3 ± 0.4 (n = 7)	1.1 ± 0.1 (n = 7)	4.5 ± 0.6 (n = 3)

* Fluorimetric and HPLC-determinations (as described in Materials and Methods) were carried out in different subcultivation charges.

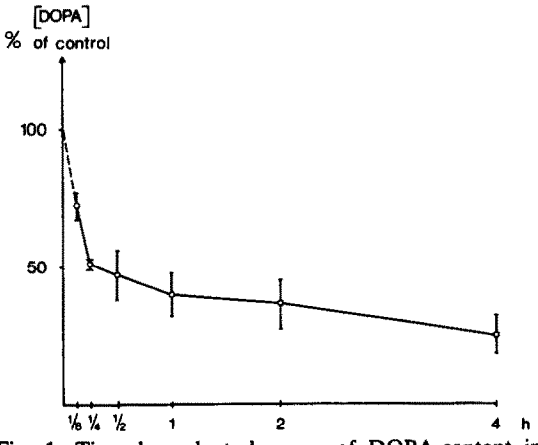

Fig. 1. Time-dependent decrease of DOPA-content in N1E-115 clone (% of control) during 4 hr of incubation with α-methyl-p-tyrosine (1×10^{-3} M).

4). The apparatus for the ion exchange procedure described by Bertler *et al.* [8] was replaced by a constant flow pump (Abimed, Düsseldorf, F.R.G., with vinyl tubes, inner diameter 0.63 mm), resulting in a more rapid and constant elution. In the eluates all compounds were analysed fluorimetrically, tyrosine after condensation with α-nitroso-β-naphthol [9], DOPA and dopamine after conversion to a dihydroxyindole [10, 11] and noradrenaline after conversion to a trihydroxyindole [12].

Since the amount of catecholamines varied from one subcultivation charge to the other, the effects of the drugs on the catecholamine levels are expressed in percent of the controls (=100%). Dopamine determined with the fluorimetric method was below the level of detection (about 5 ng/mg protein = twice the tissue blank). Therefore, high pressure liquid chromatography with subsequent electrochemical detection was used to determine the dopamine content of the cells [13, 14]. We also used high pressure liquid chromatography (HPLC) for the determination of DOPA and noradrenaline and obtained results similar to those with the fluorimetric method. HPLC resulted in a more rapid separation of the catecholamines and the subsequent electrochemical detection showed a clearly higher sensitivity.

Drugs used: α-methyl-p-tyrosine methyl ester, 3-hydroxybenzylhydrazine, dopamine and apomorphine were obtained from Sigma (München, F.R.G.). Lisuride hydrogen maleate was a gift of Dr. W. Kehr, Schering AG (Berlin, F.R.G.).

Protein assay. The pellets were dissolved in 5% NaOH at 60° and assayed for protein as described by Lowry *et al.* [15] with bovine serum albumin as a standard.

RESULTS

The tyrosine and catecholamine contents (related to mg protein) in untreated N1E-115 cells (controls) are shown in Table 1. The average amount of protein in one cultivation flask was 6.3 ± 0.2 mg S.E.M. (n = 55). Addition of 10^{-3} M α-methyl-p-tyrosine

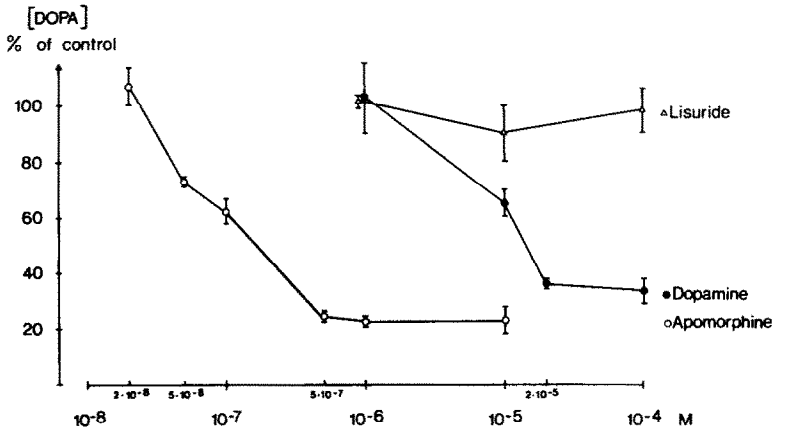

Fig. 2. DOPA-content in N1E-115 clone (% of control) after 4 hr of incubation with different concentrations of apomorphine, dopamine or lisuride.

resulted in a time-dependent decrease of the cellular DOPA content to 24.9 ± 7% S.E.M. ($n = 6$) related to the control values (=100%) after 4 hr of incubation with the drug (Fig. 1). The tyrosine content doubled during these 4 hr, dopamine and noradrenaline diminished. Addition of the DOPA-decarboxylase blocking agent 3-hydroxybenzylhydrazine (1×10^{-3} M) did not result in a further increase of the cellular DOPA content.

Figure 2 shows the dose-dependent decrease of DOPA in N1E-115 cells after 4 hr of incubation with apomorphine or dopamine (IC_{50} of apomorphine = 2×10^{-7} M, IC_{50} of dopamine = 1×10^{-5} M). The ergoline derivative lisuride hydrogen maleate had no effect on the cellular DOPA-content. None of these drugs changed the tyrosine content in the cells during the incubation period. Haloperidol up to 1×10^{-5} M did not affect the intracellular DOPA-content and could not reverse the apomorphine-induced decrease of DOPA.

DISCUSSION

The examination of the biosynthesis of catecholamines in several adrenergic clonal lines showed that the N1E-115 clone seemed to be particularly suitable for testing the regulation of catecholamine biosynthesis. This clonal line reacted with a time-dependent decrease of DOPA after the application of α-methyl-*p*-tyrosine comparable to our observations on the striatal catecholamine concentrations in rats after application of α-methyl-*p*-tyrosine [2] and led us to study several dopaminergic agonists which were effective in reversing the rigidity induced by either 6-aminonicotinamide or reserpine [1] to gain further insight into the mechanism of these drugs.

It has been shown that the N1E-115 clone contains TH and is capable of synthesising catecholamines [4, 5]. Moreover, the N1E clone also synthesises the biopterin cofactor necessary for hydroxylation of tyrosine [16]. The relatively high intracellular DOPA levels reported in this paper indicate a low AAAD-activity. This is in agreement with the observations of Waymire and Gilmer-Waymire [4] who failed to detect AAAD-activity in homogenates of N1E-115 and other neuroblastoma clones. However, there must be at least some catecholamine production in these cells, since addition of α-methyl-*p*-tyrosine did not only decrease the intracellular DOPA-content but also the dopamine and noradrenaline contents. The apparent very low level of AAAD-activity also explains the failure of 3-hydroxybenzylhydrazine—an AAAD-blocker [7]—to further increase the intracellular DOPA-content.

Four hours of incubation of the cells with the high concentration of α-methyl-*p*-tyrosine resulted in a decrease of DOPA to about one fourth of the control values due to a block of TH-activity. This decrease of DOPA after administration of a TH-blocking drug could, therefore, serve as an indirect measure of TH-activity, provided there are no changes either in the availability of tyrosine or in the catabolism of DOPA. The intracellular tyrosine content is very high so that TH works at substrate saturation [17], and none of the drugs decreased the intracellular tyrosine content. Effects of the tested drugs on the DOPA level via interference with the DOPA catabolism are improbable but cannot be excluded.

Dopamine and apomorphine, the latter in quite low doses, block the TH-activity (see Fig. 1). Richelson [17] examined the TH-activity in N1E-115 clone using the formation of [^{3}H]-OH from L-[3,5-^{3}H]tyrosine. He reported a higher K_i-value for dopamine (5×10^{-4} M). This difference to our values might be due to the shorter incubation (30 min) with dopamine. Though the dopamine agonist lisuride was highly effective in animal models [1] it had no effect on TH-activity.

Goldstein *et al.* [18] showed that the apomorphine inhibition of TH in enzyme preparations is competitive with tetrahydropteridine. Even with low cofactor concentrations of 1×10^{-7} M, apomorphine (1×10^{-6} M) had almost no effect on TH-activity. Iverson *et al.* [19] reported an IC_{50} of 1.1×10^{-5} M in lysed synaptosomes (cofactor concentration 1×10^{-3} M).

In intact synaptosomes apomorphine blocks TH-activity in much lower concentrations (IC_{50} = 2×10^{-7} M [19], 6×10^{-7} M [20]), and the action of apomorphine is partly blocked by several neuroleptics [20] enforcing the concept of a presynaptic receptor mediated control of catecholamine biosynthesis [21].

It seems premature to assign the mechanism of the apomorphine action to one of the above-mentioned possibilities, since cofactor and apomorphine concentrations in the cells are unknown. A receptor-mediated effect, comparable to the assumed presynaptic receptor in synaptosomes, seemed to be improbable, since haloperidol had no effect on the apomorphine-induced decrease of intracellular DOPA. The reported IC_{50} for the apomorphine-induced TH-blockade in the N1E-clone is, however, within the range of the IC_{50}-values obtained in synaptosomes. Moreover, it has recently been reported that lisuride had no effects on TH-activity in synaptosomes [22]. On the whole, the N1E-115 clone shows some similarity to the regulation of catecholamine synthesis in synaptosomes and might be an interesting model for studying the mechanism of TH-regulation.

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