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Toxicity of dopamine to mouse neuroblastoma × rat glioma hybrid (NG108) cells *in vitro*

WANG Ren-Gang, ZHU Xing-Zu (*Department of Pharmacology I, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

AIM: To study toxicity of dopamine to mouse neuroblastoma × rat glioma hybrid (NG108) cells. **METHODS:** Cell viability was estimated using 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. **RESULTS:** Dopamine at 100 μmol L^{-1} was toxic when added to cultures 24 h after plating. The cell viability was about 1/4 of control. Toxicity did not seem to be mediated by dopaminergic receptors because the dopaminergic antagonists sulpiride and Sch-23390 did not block the toxic effect of dopamine. Catalase 50 kU L^{-1}, superoxide dismutase 50 kU L^{-1} and *l*-ascorbic acid 200 μmol L^{-1} blocked the dopamine (125 μmol L^{-1}) toxicity and elevated the cell viability from 25.9 ± 11.0 % to 74.8 ± 4.4 %, 72.3 ± 4.5 % and 71.4 ± 2.3 %, respectively. **CONCLUSION:** Dopamine toxicity to NG108 cells was mainly attributed to the oxidation of dopamine and its toxic by-products, eg, H_2O_2.

KEY WORDS dopamine; neuroblastoma; glioma; cultured cells; cell survival; sulpiride; Sch-23390; catalase; superoxide dismutase

During ischemia, neurotransmitters such as glutamate and dopamine rise dramatically in the extracellular fluid of the brain[1-3]. Glutamate-mediated neurotoxicity to cerebral cells might involve calcium influx into cells and the formation of nitric oxide[4,5]. Oxygen free radicals originating from dopamine oxidative processes might be a cause of the degeneration of the dopaminergic nigrostriatal cells[6]. In the present study, we observed the toxicity of dopamine to mouse neuroblastoma × rat glioma hybrid (NG108) cells and the protective role of oxygen free radical scavengers catalase and superoxide dismutase.

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MATERIALS AND METHODS

Reagents Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were purchased from Gibco. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was obtained from Serva. Dopamine, sulpiride, *l*-ascorbic acid, catalase, and superoxide dismutase were purchased from Sigma. Sch-23390 was from Schering Corp, Bloomfield NJ, USA.

NG108 cell culture NG108 cells, obtained from Dr De-Maw CHUANG (National Institute of Mental Health, Bethesda MD, USA), were cultured in a growth medium consisting of DMEM 90 % and heat-inactivated FCS 10 % supplemented with penicillin 100 kU L^{-1} and streptomycin 100 mg L^{-1} in a humidified atmosphere of 95 % air + 5 % CO_2 at 37 ℃. The cells were grown in 24-cm^2 glass bottles. Cell viability was assessed by trypan blue dye exclusion. The medium was frequently renewed to avoid exposure of the cells to an acidic environment.

Measurement of cell viability The MTT assay was used to measure the viability of cells[7]. Cells were placed in 96-well microtitre plates with a density of 1.8×10^4 cells per well and incubated with normal medium for 24 h. The medium was replaced with new medium including drugs but excluding FCS. The cells were incubated for a further 24 h. After the medium was aspirated off, the cells were incubated with normal medium for 1 h. The medium 100 μL with MTT 0.5 g L^{-1} was added to each well and incubated at 37 ℃ for 4 h. Me_2SO 100 μL was added to dissolve the MTT formazan for 15 min. The optical density at

550 nm was determined by a DG3022 plate reader (Chinese Electron Tube Factory, Nanjing, China). Cell viability was expressed as MTT reduction in comparison with control.

Statistic analysis Data were expressed as $\bar{x} \pm s$ and analyzed by t test.

RESTULTS

Cultures were exposed to dopamine 2.5, 25, 125, or 250 μmol L^{-1} for 24 h. No effect of dopamine was seen at 2.5 and 25 μmol L^{-1}. In dopamine 125 μmol L^{-1}, the cell viability fell to about 1/4 of control. No cells survived at dopamine 250 μmol L^{-1} at which phenol red acquired a brownish color, presumably from the presence of oxidative products of dopamine (Fig 1).

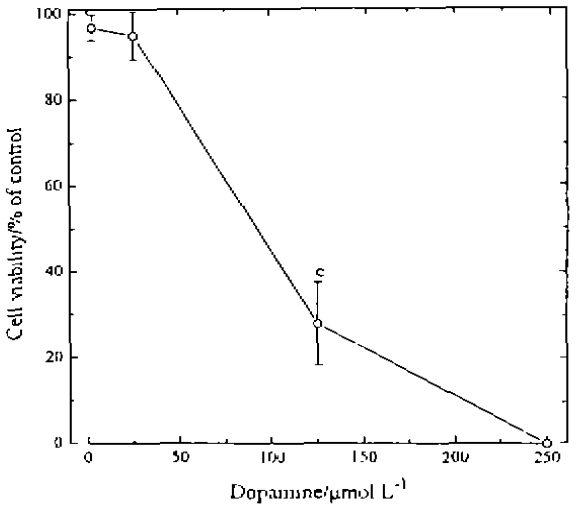

Fig 1. NG108 cells' viability in response to dopamine treatment. $n=7$ samples, $\bar{x} \pm s$.
$^cP<0.01$ vs control.

Sulpiride and Sch-23390 failed to block the toxic effect of dopamine even at a very high concentration of 200 μmol L^{-1} (Tab 1).

When cultures were exposed to dopamine 125 μmol L^{-1}, dopamine 125 μmol L^{-1} plus catalase 50 kU L^{-1}, or dopamine 125 μmol L^{-1} plus superoxide dismutase 50 kU L^{-1}, the viability of NG108 cells was increased greatly by catalase and superoxide dismutase. l-Ascorbic acid at 200 μmol L^{-1} also blocked dopamine toxicity (Tab 1).

Tab 1. Effects of sulpiride (200 μmol L^{-1}), Sch-23390 (200 μmol L^{-1}), catalase (50 kU L^{-1}), superoxide dismutase (50 kU L^{-1}), and l-ascorbic acid (200 μmol L^{-1}) on dopamine (125 μmol L^{-1}) toxicity to NG108 cells. $n=7$ samples.
$^cP<0.01$ vs control; $^fP<0.01$ vs dopamine.

Drugs	Cell viability/% of control
sulpiride	102.6±6.9
Sch-23390	101.3±6.3
catalase	104.1±14.2
superoxide dismutase	103.9±8.1
l-ascorbic acid	98.2±5.6
dopamine	25.9±11.0^c
dopamine+sulpiride	27.3±8.6
dopamine+Sch-23390	24.0±6.4
dopamine+catalase	74.8±4.4^f
dopamine+superoxide dismutase	72.3±4.5^f
dopamine+l-ascorbic acid	71.4±2.3^f

DISCUSSION

In the present study, we found that dopamine was toxic in 100 μmol L^{-1} to NG108 cells and that the toxicity of dopamine was not blocked by dopaminergic antagonists sulpiride and Sch-23390. These data argue against receptor mediation of dopamine toxicity. The possibility that dopamine toxicity might be mediated through toxic by-products of the oxidation of dopamine was considered. The results that catalase and superoxide dismutase protected NG108 cells against dopamine toxicity suggest that H_2O_2 and superoxide anion mediate the dopamine toxicity. It has been reported that the oxidative metabolism of dopamine has the potential to generate cytotoxic free radicals such as H_2O_2[8]. H_2O_2, in turn, can give rise to the formation of hydroxyl radical and other reactive species which may lead to tissue damage. Our results suggest that a likely mechanism for dopamine toxicity

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to NG108 cells is the oxidation of dopamine and the production of H_2O_2.

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多巴胺对小鼠神经母细胞瘤和大鼠神经胶质瘤的杂交(NG108)细胞在体外的毒性

王仁刚，朱兴旋 R965.2

(中国科学院上海药物研究所，上海 200031，中国)

目的：研究多巴胺（DA）对小鼠神经母细胞瘤和大鼠神经胶质瘤的杂交细胞 NG108的毒性. **方法**：用 MTT 法测定 NG108细胞的活性. **结果**：当 DA 浓度在 100 μmol L^{-1}时对 NG108细胞有毒性作用. 这时的细胞活性仅为对照的 1/4左右. DA 受体拮抗剂舒必利和 Sch-23390不能阻断 DA 毒性，表明 DA 对 NG108细胞的毒性作用不是由 DA 受体介导的. DA (125 μmol L^{-1})的毒性作用能被过氧化氢酶 (50 kU L^{-1})、超氧化物歧化酶 (50 kU L^{-1})和维生素 C (200 μmol L^{-1})抑制. 它们分别能使 NG108细胞的活性由 DA 单独作用时的 25.9±11.0 %上升到 74.8±4.4 %、72.3±4.5 %和 71.4±2.3 %. **结论**：DA 对 NG108细胞的毒性作用是由 DA 氧化代谢产生的有毒产物如过氧化氢引起的.

关键词 多巴胺；神经母细胞瘤；神经胶质瘤；培养的细胞；细胞存活；舒必利；Sch-23390；过氧化氢酶；超氧化物歧化酶