

Desipramine and fluoxetine antagonized 5,7-dihydroxytryptamine-induced lesion on rat hippocampal and cortical neurons

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KEY WORDS 5,7-dihydroxytryptamine; desipramine; fluoxetine; hippocampus; cerebral cortex; serotonin; neurons; cultured cells

ABSTRACT

AIM: To assess the protective effect of desipramine (Des) and fluoxetine (Flu) on the neurons against the lesion induced by a selective serotonergic neurotoxin *in vitro*. **METHODS:** The 10-day cultured primary neurons of hippocampus and cortex of rat was exposed to 5,7-dihydroxytryptamine (5,7-DHT) to determine the optimal lesion concentration and duration. Before exposing to 5,7-DHT, Des and Flu was added to the medium for 30 min to observe the protective effects. **RESULTS:** The optimal concentration and duration for 5,7-DHT was $600 \mu\text{mol} \cdot \text{L}^{-1}$ and 4 h, respectively. Both Des and Flu showed a protective effect in the dose range of $0.8 \mu\text{mol} \cdot \text{L}^{-1}$ to $10 \mu\text{mol} \cdot \text{L}^{-1}$ and $0.04 \mu\text{mol} \cdot \text{L}^{-1}$ to $0.6 \mu\text{mol} \cdot \text{L}^{-1}$, respectively, when the neurons were injured by 5,7-DHT $600 \mu\text{mol} \cdot \text{L}^{-1}$ for 4 h. Flu showed a higher efficacy than Des. Both exhibited a more powerful protective effect on the hippocampal neuron than on the cortical neuron. **CONCLUSION:** The antidepressant effect of Des and Flu was attributed to their protective effect on the injured serotonergic neuron of the hippocampus and the cortex.

INTRODUCTION

The dysfunction of the central serotonergic (5-HT) systems was implicated in the pathophysiology of many

neuropsychiatric disorders including depression and anxiety^[1]. The predominant theory was that a lack of sufficient serotonergic activity contributed to depression. This notion came about with the awareness that many antidepressant medications were effective blockers of either the enzymatic oxidation or the reuptake of serotonin^[2]. The hypothesis also appeared to be consistent with the evidence that depression was often associated with decreased levels of the metabolites of 5-HT in the cerebrospinal fluid^[2]. However, reports upon effects of antidepressants on densities and mRNA level of 5-HT receptor were not always consistent. The exact mechanism of depression and antidepressants, therefore, was awaited to be further studied. 5,7-DHT, a selective neurotoxin of 5-HT neuron, induced a hyposerotonergic activity by combined with the uptake site of 5-HT and/or disturbed the combination of 5-HT with a serotonin binding protein (SBP) to exhaust the 5-HT of synapse^[3]. The serotonergic activity change induced by 5,7-DHT was similar to that of depression. It had been demonstrated that 5,7-DHT was useful in assessing the physiological function of 5-HT in the hippocampus *in vivo*^[4], but there were few studies on evaluating the serotonergic function *in vitro*. Our research focused on the establishment of a model *in vitro* for assessing the serotonergic function, and the protective effect of antidepressants on injured serotonergic neurons, to provide screening means for antidepressants and evidences for exploring the action mechanism.

MATERIALS AND METHODS

Rats Neonatal Wistar rats (0-1 d, Grade II, Certificate No 01-3039 from Beijing Supervising Committee for Medical Experimental Animals) were supplied by the Experimental Animal Center, Academy of Military Medical Sciences.

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Drugs 5, 7-Dihydroxytryptamine (5, 7-DHT, Sigma), desipramine hydrochloride (Des, Sigma), fluoxetine hydrochloride (Flu, Sigma).

Preparation of the cultured neurons

Primary neuronal cultures were generated essentially as described by Weiss *et al*^[5] with some modification. Briefly, cortical and hippocampal structures were removed from neonatal Wistar rats (0–1 d), enzymatic digestion (0.125 % trypsin) at 37 °C for 30 min, and mechanically dissociated with a fire-narrowed Pasteur pipette, and plated (1×10^6 cells \cdot L⁻¹) in 96-well Costar culture dishes previously coated with L-poly-lysine (0.1 %, $M_r = 150 - 300$, Sigma). The plated medium was the mixture of Dulbecco's modified Eagle's medium (DMEM, high glucose), glutamine 100 mg \cdot L⁻¹, 10 % heat-inactivated horse serum and fetal bovine serum, respectively. After 24 h, cells were seeded in the maintenance medium, which was the mixture of DMEM (high glucose), glutamine 100 mg \cdot L⁻¹, 1 % heat-inactivated horse serum and 1 % N₃ nutrient that included insulin 10 mg \cdot L⁻¹, transferrin 200 mg \cdot L⁻¹, progesterone 40 nmol \cdot L⁻¹, putrescine 200 μ mol \cdot L⁻¹, hydrocortisone 10 mg \cdot L⁻¹, 3,3',5'-triiodo-thyronine 20 mg \cdot L⁻¹, selenium salt 6 nmol \cdot L⁻¹ and bovine serum albumin (BSA) (0.001 %) (all from Sigma). The culture medium was half-refreshed twice a week until 10 d (refreshing for three times).

Determination of optimal lesion concentration of 5,7-DHT and lesion duration Previously cultured cortical and hippocampal neurons were exposed to noted concentrations of 5, 7-DHT for various durations. The injured neurons were recovered in the maintenance medium for two days. Prior to the measurement, cultures were incubated in the serum-free DMEM media for 1 d. According to Tada *et al*^[6] method of measuring the cell vitality, after 4 h the cultures exposed to 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium (MTT) 0.42 g \cdot L⁻¹, the supernatant was removed thoroughly, and 10 % sodium dodecyl sulfate-0.01 mol \cdot L⁻¹ HCl (SDS) was added to break the cells and solve the MTT derivatives for 16 h in CO₂ incubator. With two-wavelengths (570 nm and 630 nm), the absorption values of each well were determined by Multiskan MCC/340 MK II.

Protective effect of antidepressants on the injured neurons After 30 min the 10-day-cultured neurons exposed to noted concentrations of Des and

Flu, 5, 7-DHT 600 μ mol \cdot L⁻¹ was added to the medium to lesion for 4 h, the rest procedure was the same as above.

Data analysis All results were subjected to group *t* test to determine whether processed group data were significantly different from the lesion control and/or the vehicle control ($P < 0.05$).

RESULTS

Morphological observations The initial manifestation of 5, 7-DHT-induced lesion of neurons were membrane wrinkling, and withering of the cell bodies into dots and of the dendrites and axons into thin threads with the duration lasted longer. Part of the cells broke away from the culture wall. The cell bodies of the vehicle control and the protected neurons were big and full with more and stronger dendrites and axons, which demonstrated a more stereoscopic image.

Optimal lesion concentration and duration Absorption values were the vitality index of the neurons injured by 5, 7-DHT (Tab 1). Combined with morphological observation, the lesion concentration and duration were selected as 600 μ mol \cdot L⁻¹ and 4 h, respectively.

Protective effect of antidepressants The absorption values of the neuron protected by antidepressants from 5, 7-DHT lesion were shown in Tab 2. Both Des and Flu had significant protective effects on the injured neurons ($P < 0.01$), but Flu possessed a higher efficacy than Des. A more powerful protective effect on the hippocampal neuron was exhibited.

DISCUSSION

5, 7-DHT selectively destroyed serotonergic neurons following various routes of administration^[4]. It rapidly accumulated in target neurons and inhibited the reuptake of 5-HT, indicating that the selectivity of 5, 7-DHT was based on the activity of high-affinity processes located on serotonergic neurons. Baumgarten^[7] reported that the affinity of 5, 7-DHT for 5-HT and norepinephrine (NE) uptake differed by a factor of about 16. The difference in the affinity of the two transport systems was sufficiently large to explain why central 5-HT neurons were always more profoundly

**Tab 1. Lesion effect of various concentrations of 5, 7-DHT in different durations on the cultured cortical and hippocampal neurons. $n = 6$ of triplicate measurements of two independent experiments. $\bar{x} \pm s$.
^b $P < 0.05$, ^c $P < 0.01$ vs control.**

Conc/ $\mu\text{mol} \cdot \text{L}^{-1}$		Time/h				
		1	2	4	8	22
Control	Hippocampus					0.70 ± 0.06
	Cortex					0.49 ± 0.11
100	Hippocampus	0.64 ± 0.06	0.62 ± 0.06	0.79 ± 0.09	0.54 ± 0.05	0.82 ± 0.15
	Cortex	0.43 ± 0.03	0.59 ± 0.03	0.62 ± 0.22	0.55 ± 0.12	0.45 ± 0.07
200	Hippocampus	0.75 ± 0.11	0.70 ± 0.07	0.82 ± 0.06	0.67 ± 0.19	0.85 ± 0
	Cortex	0.45 ± 0.04	0.50 ± 0.07	0.47 ± 0.05	0.57 ± 0.05	0.41 ± 0.03
400	Hippocampus	0.72 ± 0.06	0.77 ± 0.12	0.64 ± 0.13	0.59 ± 0.16	0.47 ± 0.13
	Cortex	0.44 ± 0.02	0.58 ± 0.04	0.41 ± 0.18	0 ^c	0.11 ± 0.08^c
600	Hippocampus	0.64 ± 0.04	0.74 ± 0.04	0.81 ± 0.20	0.90 ± 0.01	0.38 ± 0.01^b
	Cortex	0.57 ± 0.07	0.54 ± 0.02	0.04 ± 0^c	0 ^c	0 ^c
800	Hippocampus	0.32 ± 0.27^b	0.59 ± 0.03	0 ^c	0.07 ± 0.06^c	0.53 ± 0.07
	Cortex	0.29 ± 0.05^b	0.16 ± 0.07^c	0 ^c	0 ^c	0 ^c
1000	Hippocampus	0.10 ± 0.10^c	0 ^c	0 ^c	0.08 ± 0.05^c	0 ^c
	Cortex	0.45 ± 0.07	0.02 ± 0.04^c	0 ^c	0 ^c	0 ^c

**Tab 2. Protective effect of Des and Flu on the cultured hippocampal and cortical neurons injured by 5, 7-DHT. $\bar{x} \pm s$. $n = 6$ from triplicate measurements of two independent experiments.
^a $P > 0.05$, ^b $P < 0.05$, ^c $P < 0.01$ vs lesion control.**

Conc/ $\mu\text{mol} \cdot \text{L}^{-1}$	Desipramine		Conc/ $\mu\text{mol} \cdot \text{L}^{-1}$	Fluoxetine	
	Hippocampus	Cortex		Hippocampus	Cortex
10	0.33 ± 0.03^c	0.20 ± 0.08^c	0.6	0.37 ± 0.18^c	0.27 ± 0.15^c
8	0.42 ± 0.09^c	0.18 ± 0.03^c	0.4	0.40 ± 0.26^c	0.24 ± 0.10^c
6	0.36 ± 0.05^c	0.17 ± 0.05^c	0.2	0.55 ± 0.24^c	0.24 ± 0.18^c
4	0.34 ± 0.05^c	0.12 ± 0.05^b	0.1	0.35 ± 0.02^c	0.28 ± 0.12^c
2	0.32 ± 0.12^c	0.08 ± 0.03^a	0.08	0.37 ± 0.04^c	0.20 ± 0.07^c
1	0.20 ± 0.09^c	0.015 ± 0.026^a	0.06	0.27 ± 0.06^c	0.17 ± 0.03^c
0.8	0.13 ± 0.11^c	0.003 ± 0.008^a	0.04	0.19 ± 0.08^c	0.06 ± 0.04^a

Hippocampal lesion control 0.02 ± 0.04 ($n = 18$)
 Cortical lesion control 0.02 ± 0.04 ($n = 15$)

affected by 5, 7-DHT than were NE neurons. This was consistent with our experiment. Fluoxetine, a selective serotonin reuptake inhibitor, provided a more powerful protective effect on the injured hippocampal and cortical serotonergic neuron by 5, 7-DHT than Des. This suggested that the antidepressive effect of Des and Flu be related to binding to the reuptake site of monoamine, thus affect the monoamine levels. We presume that there are some intrinsic serotonin analogues, including serotonin metabolites such as 5-hydroxyindoleacetic acid, and indolenine, and so on, may produce toxic effect on the neurons with the

serotonergic reuptake site by the similar mechanism to 5, 7-DHT in the brain of depressive patients due to delayed-clearance for some reasons, though it needs support from experimental evidences.

The majority of recent functional brain imaging studies have demonstrated a reduction in neuronal activity most pronounced in the left prefrontal cortical and limbic areas, particularly in recurrent or chronic depressive disorders^[8]. This reduction in neuronal activity is evidenced by decreased blood flow and glucose utilization in these neuroanatomical areas. George and colleagues^[9] have used functional brain

imaging to study normal individuals experiencing transient alterations of normal affective states such as sadness and happiness. In contrast to the deficiencies in the frontal and limbic blood flow observed in chronically depressed individuals, an activation of these same circuits occurs during self-induced sadness. Although alternative explanations exist, one heuristic model may be that stressors resulting in dysphoria in normal individuals activate this specific anatomical circuitry which, when persists, results in depression and with further persistence and/or recurrence may eventually produce the burnout or inactivation of this same circuitry^[9]. The decreased blood flow may result in a delayed-clearance of intrinsic neurotoxins and induce the reduction in the neuronal activity.

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In conclusion, this model *in vitro* is a simple and useful means for evaluating and screening agents acting on the serotonergic neurons. Des and Flu have protective effect on injured serotonergic neurons by 5,7-DHT, which may be one of their antidepressant mechanisms.

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地昔帕明和氟西汀拮抗 5,7-二羟色胺
对海马和皮质神经元的损伤

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关键词 5,7-二羟色胺; 地昔帕明; 氟西汀;
海马; 大脑皮质; 血清素; 神经元; 培养的细胞

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目的: 评价抗抑郁剂地昔帕明和氟西汀对 5,7-二羟色胺损伤神经元的拮抗作用. 方法: 原代培养 10 d 的大鼠海马和皮层细胞加入不同浓度的地昔帕明和氟西汀作用 30 min 后, 用 5,7-二羟色胺 600 μmol·L⁻¹ 损伤 4 h, MTT 法结合形态学观察判断药物的保护作用. 结果: 地昔帕明和氟西汀对神经元损伤具有显著拮抗作用, 剂量范围分别为 0.8-10 μmol·L⁻¹ 和 0.04-0.6 μmol·L⁻¹. 结论: 地昔帕明和氟西汀对 5,7-二羟色胺的神经元损伤具有显著拮抗作用, 氟西汀效价高于地昔帕明(约一个数量级). 两种抗抑郁剂对神经元的保护作用可能是它们抗抑郁作用的机制之一.

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