EFFECT OF HALOPERIDOL ON THE NUMERICAL DENSITY OF NEURONS AND NUCLEAR HEIGHT IN THE RAT HIPPOCAMPUS

(A stereological and histopathological study)

B. Unal[1], M. E. Özbek[1], M. D. Aydın[2], N. Aydın[3], Z. Bulucu[1], Ö. Vuraler[1], E. Odaci[4], B. Sahin[4], S. Kaplan[4 CA]

Depts. of [1]Histology & Embryology, [2]Neurosurgery, [3]Psychiatry, Medical Faculty, Atatürk University, Erzurum; Depts. of [4]Histology & Embryology and Anatomy, Medical Faculty, Ondokuz Mayis University, Samsun, Turkey
Corresponding author; E-mail: skaplan@omu.edu.tr

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SUMMARY

In recent studies, some neuroleptics have shown neurotoxic activities. Clinical and experimental studies have been carried out to investigate the effects of different neuroleptic drugs considered to affect the central nervous system. The aim of this study was to evaluate neurotoxic effects of haloperidol on hippocampal neurons. The drug was given in daily doses of either 1 or 3 mg/kg for 6 weeks to adult male guinea pigs. After treatment, all animals were anaesthetized via short inhalation of ether, and then were fixed by a mixture of 2% glutaraldehyde and +2% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed from the cranium and stored in the same fixative overnight. On the following day, the CA1 region of the hippocampus was dissected out. After embedding in araldite resin and obtaining semi-thin sections, the tissues were stained with toluidine blue. The physical disector was used for measurements of nuclear height and numerical density of neurons and the sections were also evaluated histopathologically. The numerical density of neurons and nuclear height in the hippocampus for the low-dose (1 mg/kg) and high-dose (3 mg/kg) experimental groups were 12.4 mm ^{3} and 3.6 μm and 7.14 mm ^{3} and 3.56 μm, respectively. In contrast, the control group had a neuronal numerical density of 16.55 mm^{-3} and a nuclear height of 4.09 μm. There was a significant difference in both the mean density of neurons and the mean height of nuclei between haloperidol-treated and control groups ($p<0.05$). There was also a statistical difference in the mean density of neurons (but not in nuclear height) when comparing the dosage of haloperidol ($p<0.05$). These findings suggest that haloperidol treatment may lead to a loss of neurons as well as a decrease in the height of nuclei in the hippocampus.

KEY WORDS: haloperidol; hippocampus; stereology; neuron density; nuclear height

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INTRODUCTION

Neurons in the central nervous system vary in their susceptibility to the toxic side effects of drugs. The extent of damage depends on the duration and/or dose of the drug, as well as other uncounted factors. The hippocampus (especially the CA1 region), which is an essential component of learning and memory processes (3,4,5,6), is exceptionally vulnerable to necrotic damage (1,2). This region has been shown to be damaged by a number of toxicants (2,7,8) and/or toxic effects of some neuroleptics (9,10). Clinical (11,12) and experimental (13) studies have been carried out to investigate the effects of different neuroleptic drugs considered to affect the central nervous system (14).

Haloperidol is known as a dopamine receptor antagonist and a sigma receptor-active neuroleptic (15). It is a potent antipsychotic frequently used in patients with schizophrenia (16). Although a lot of useful information has been obtained from structural (17), ultrastructural (18), quantitative (19,20) and clinical (11) studies, there are few stereological studies evaluating the effects of haloperidol treatment (20-23).

Modern stereological techniques comprise a set of simple and efficient rules for the quantitative estimation of three-dimensional structure from two-dimensional sections (24). These estimations are based on geometric statistics and require no assumptions to be made about the shape or orientation of structure (25). The physical disector was developed as an unbiased and efficient method to estimate neuron number in a region. This tool gives a reliable estimation of particle number and size in an anatomically defined area (24,26). Therefore, the quantitative analyses of neurons become very significant finding in these kind of studies when assessing neuron proliferation or degeneration following drug application or ischemia (14,27). It is also useful to examine structures that require an assessment of changes in the number of objects as an indicator of therapeutic effectiveness (28).

In the present study, we have attempted to determine the effect of haloperidol-treatment on the numerical density of neurons and the height of the nucleus in the rat hippocampus. We have used the physical disector counting method (24) on semi-thin sections at the light microscopy level in order to make such measurements, and have also tried to assess histopathological and structural changes on the same sections.

MATERIAL AND METHODS

Experimental design and applying the drug: In the present study fifteen adult male guinea pigs were used. All animals were maintained under standardized conditions of light (12 hours light/12 hours dark) and temperature. The experimental animals were divided into 3 groups (5 pigs per group). Animals were treated daily for 6 weeks according to the following schedule; I) haloperidol 1 mg/kg, i.p. (low-dose group); II) haloperidol 3 mg/kg, i.p. (high-dose group); III) saline vehicle, i.p. (control group). This study was approved by the Ethical Committee of Atatürk University.

Perfusion and fixation: Following the drug-treatment, all animals were anaesthetized via short inhalation of ether, and then perfused intracardially. Initially, a 0.9% saline (30 ml) solution was given followed by a mixture of 2% paraformaldehyde+2% glutaraldehyde (150 ml) in 0.1 M phosphate buffer, pH 7.4 for approximately 30 minutes, at room temperature. Brains were removed and stored in the same fixative overnight at 4°C (30). On the following day, the hippocampus in each brain was dissected out as described previously (31), and the CA1 region of the hippocampal tissue was embedded in resin.

Microscopy for stereological analysis and histopathological examination: The hippocampal tissue samples were post fixed in 1% osmium tetroxide for 1 h, dehydrated through a graded alcohol series, and embedded in Epon resin. Each resin-embedded sample was cut into 1μm serial sections with a Nova LKB Bromma ultratome. The selection of the physical disector pairs was done as described by Sterio (24). We obtained approximately 160 sections from each hippocampus. Based on the pilot study, pairs from every 4th section were chosen randomly, and in this way approximately 15-20 section pairs were obtained. This number is in an acceptable range for stereological analysis (32-34). Disector pairs were taken from the tissue at a known interval, until the tissue sample was exhausted. Two consecutive sections were mounted on each slide. Photographs of adjacent sections were taken with a digital camera at a magnification of X400. Nucleoli of neurons seen in the reference section but not in the look-up section were counted. To increase the countable particle number, i.e. nucleoli, we exchanged the role of sections in the second step. An unbiased counting frame was placed on the reference and the look-up sections on the screen of the PC to perform the counting according to the disector counting method. The bottom and the left hand edges of the counting frame are considered to be the forbidden (exclusion) lines together with the extension lines. Other boundaries of the frame and the top-right corner were considered to be inclusion points and any particle that hit these lines or was located inside the frame was counted as a disector particle (35) (Fig. 1).

The mean numerical density of neurons ($N_{v(neuron)}$) per μm^3 was estimated using the following formula (24,36).

$$Nv(neuron) = \sum Q^{-}(neu) \,/\, t \times A$$

Where $\Sigma Q^{-}_{(neu)}$ is the total number of nucleoli counted in the reference section; t is the mean section thickness (1μm), and A is the area of the unbiased counting frame.

The mean nuclear height ($H_{(nucleus)}$), which is a measure of the size of nucleus that depends on the section plane, was estimated by the following equation (24).

$$H(nucleus) = \left(\frac{\sum Q(nucleus)}{\sum Q^{-}(nucleus)} \right) \times t$$

Where $\Sigma Q_{(nucleus)}$ is the total number of nuclei counted in the reference section, $\Sigma Q^{-}_{(nucleus)}$ is the total number of disector nuclei counted in the reference section, and t is the mean section thickness (1μm).

Finally, histopathological examinations were carried out on images of the same sections.

Statistical analysis: To evaluate the significance of observed differences, we used the Student's t test (two tailed, significance limit is p=0.05 in this test). All statistical calculations were performed using SPSS v10.0 for Windows.

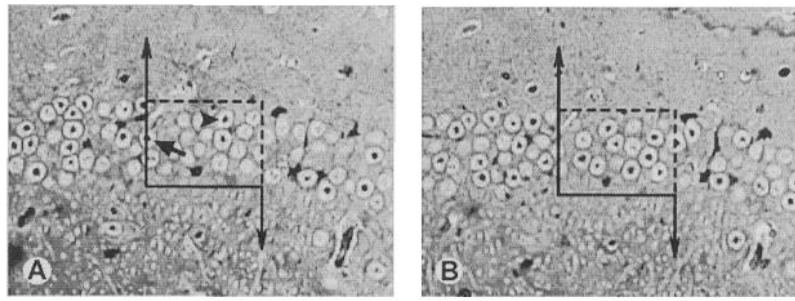

Figure 1: An application of the physical disector counting method. The same areas of two adjacent sections, separated by 1 μm, are shown (A, B). Nucleoli hitting the exclusion lines were excluded (arrow) from counting. Nucleoli hitting the inclusion lines or located inside the frame (arrow head) were counted as disector particles if their profiles are not seen in the look-up section (B), x 1000.

RESULTS

STEREOLOGICAL RESULTS

Mean numerical density of neurons ($N_{v(neuron)}$): The mean numerical density of neurons for both the haloperidol-treated experimental groups and the control group are shown in Table I. We observed that the neuronal densities of the experimental groups were significantly decreased in comparison to the control subjects. Specifically, the mean neuronal densities in the low-dose and high-dose groups were reduced by 26.65% and 56.86%, respectively, relative to the control group ($P<0.05$). There was also a statistical difference in neuronal density between the low- and high-dose groups ($P<0.05$).

Table I: The estimation of numerical density of neurons and nuclear height of neurons in the CA1 region of the rat hippocampus

	Control Group (saline) (n=5)	Low-Dose Group (1 mg/kg/day haloperidol) (n=5)	High-Dose Group (3 mg/kg/day haloperidol) (n=5)
Mean Neuronal Density (μm^3)	16.55 ±1.599	12.14±1.337* ‡	7.14±0.877*
Mean Nuclear Height (μm)	4.09±0.458	3.60±0.378*	3.56±0.349*

Mean ±SEM, *Drug-treated vs control, P<0.05; ‡ Drug-treated vs drug-treated, P<0.05.

Mean nuclear height ($H_{(nucleus)}$): Mean nuclear height for the experimental and control groups are also shown in Table I. There were significant differences between the haloperidol-treated groups and the control group (P<0.05). The relative decrease in mean nuclear height in the low-dose (P<0.05) and the high-dose (P<0.05) experimental groups, in comparison to the control group, were 13.61% and 14.89%, respectively. However, there was no dosage-dependent statistical difference in nuclear height with haloperidol treatment 1.12%; P>0.05).

HISTOPATHOLOGICAL RESULTS

A morphological analysis was made of the hippocampal neurons under each of the experimental conditions described previously (Fig 2). In particular, the nuclear chromatin of haloperidol-treated samples appeared swollen and clumped, suggestive of necrosis. There was also evidence of chromatin condensation and nuclear shrinkage. In terms of haloperidol dosage, the number of necrotic neurons increased significantly in the high-dose group relative to the low-dose group.

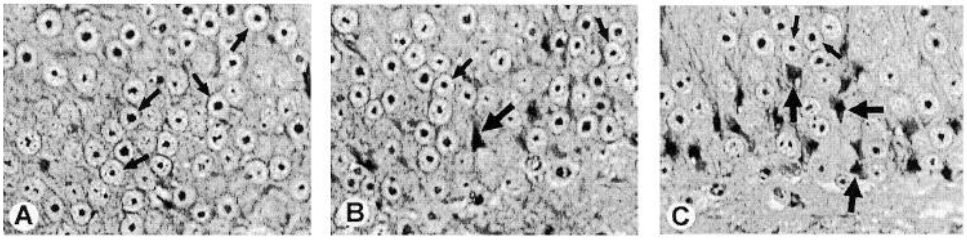

Figure 2: Light micrographs of the hippocampus of guinea pigs. (A) Neuronal cell nuclei (thin arrows) in the control group, illustrating apparently normal ultrastructural morphology. (B) A considerable number of degenerated neurons (thick arrows) were observed in the low-dose experimental group. (C) In the high-dose experimental group, there was an increase in the number of necrotic neurons (thick arrows). Both the nucleus and the cell body of neurons were shrunken and had lost much of their morphologic detail. The number of intact neurons (thin arrows) in this high-dose group was decreased in comparison to the control group (A), x 2667.

DISCUSSION

In this study, we investigated the effects of different doses of haloperidol on the numerical density of neurons and height of nuclei in the hippocampus using the physical disector counting method (24). Morphological studies have suggested that neuroleptics act as neuroprotective agents by stimulating neurogenesis (29,37). However, it has also been reported that treatment with these agents may have side effects, such as neurodegeneration of the hippocampus, striatum, and medial prefrontal cortex (9,38). The present study was limited to a 42-day period of treatment. In rats, a 42-day chronic treatment period corresponds to about 6 years of treatment in patients (29). Therefore, the results of this study may imitate chronic haloperidol treatments in humans.

Dawirs and co-workers have suggested that a low-dose treatment with haloperidol increases cell proliferation in the dentate gyrus (37) but we could not find any mitotic figures in the CA1 region of the hippocampus. When the low- and high- dose haloperidol-treated groups were examined by the physical disector counting method, the numerical density and nuclear height of hippocampal neurons were decreased significantly in comparison to the control group. The mechanism of haloperidol-induced cell death is not well understood. This drug may activate cholinergic pathways (16) that result in memory dysfunction (39). This might also be associated with an increase in p53 expression (10). Histopathological results showed that there are many neurons that have an abnormal structure in sections of the drug-treated animals. In these neurons, we observed clumping, condensation of chromatin, and shrinkage and peripheral displacement of the nucleus. Finally, the cell shape was changed from concave to convex.

In conclusion, the results of our present study have suggested that there is a relationship between haloperidol treatment and a reduced numerical density of hippocampal neurons. Our results are consistent with results of Andreassen and co-workers in that haloperidol causes neuronal degeneration and a decrease in the number of neurons (21). It may be concluded that haloperidol is a toxic substance to neurons, and it is possible that a cognitive impairment might be expected as a result of hippocampal neuron loss after the high-dose chronic haloperidol treatment. Consequently, in clinical settings, a high-dose neuroleptic treatment with haloperidol should be avoided.

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