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EFFECTS OF COLCHICINE ON OXYTOCIN- AND VASOPRESSIN-CONTAINING NEURONS IN THE SUPRAOPTIC NUCLEUS OF RATS OF DIFFERENT AGES

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ABSTRACT

The mitotic inhibitor colchicine inhibits the axonal flow of proteins in various types of neurons. A differential effect of colchicine on vasopressin-containing neurons compared to oxytocin-containing ones has been suggested, and a decline in vasopressin immunoreactivity in the neurohypophysial system of aged monkeys has been reported. In the present work, we examined the action of colchicine in 3- and 15-month-old rats. Treatment with colchicine decreased the number of oxytocinergic neurons in 3-month-old and 15-month-old rats by 9.5% and 11.1%, respectively. There was also a 30.1% decrease in the number of vasopressinergic neurons in untreated rats after 15 months. These results show that oxytocinergic and vasopressinergic neurons of the supraoptic nuclei are differentially affected by colchicine and aging.

Key words: Aging, colchicine, supraoptic nucleus, vasopressin, oxytocin, rats

INTRODUCTION

The mitotic inhibitor, colchicine, inhibits the axonal flow of proteins in various types of neurons [6,7,15,18]. Thus, in the hypothalamic-neurohypophysial tract of the rat, colchicine almost completely blocks the transport of neurosecretory material [1,11,22]. Hindelang-Gertner *et al.* [16] observed that colchicine affected the secretory neurons of the neurohypophysial system, and led to the preferential accumulation of dense material in vesicles of the supraoptic and paraventricular nuclei.

The magnocellular neurons of the supraoptic nucleus (SON) of the hypothalamus are involved in the synthesis, transport and release of vasopressin and oxytocin [33,37,38,41]. Parish and Pickering [23] suggested a differential effect of colchicine on these two neuronal systems, with vasopressin-containing neurons being affected for a longer period than oxytocin-containing ones. Sladeck *et al.* [29] reported a decline in the immunoreactivity of vasopressin-containing neurons in the neurohypophysial system of the aged monkey. Similarly, in Fisher 344 rats, there was a decrease in the immunoreactivity of vasopressin with aging while oxytocin levels were maintained [30].

In the present work, we examined the effect of colchicine on oxytocinergic and vasopressinergic neurons in the supraoptic nuclei of rats of different ages. The oxytocin and vasopressin immunoreactive neurons in the central portion of the supraoptic nucleus were counted after colchicine injection into the lateral ventricle of 3- and 15-month-old rats.

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MATERIAL AND METHODS

Forty male Wistar rats were housed two per cage at 21 ± 2°C on a 12 h light-dark cycle (lights at 7:00 a.m.) with free access to food and water. Rats of two age groups (3 months old, n=24, and 15 months old, n=16) were used. Twelve of the three-month-old rats were used to examine oxytocin immunoreactivity (OX-IR) and the other 12 were used for vasopressin immunoreactivity (VAS-IR). Half of the animals (n=6) in each of these subgroups received colchicine injections in the lateral ventricle. The same procedure was used for the 15-month-old rats.

For colchicine injection, the rats were anestethized with 3.5% chloral hydrate (1 ml/100 g of body weight, i.p.) and then fixed in a stereotaxic device prior to the administration of colchicine (50 µg in 25 µl saline) into the lateral ventricle. The animals were killed 48 h later with a lethal dose of 35% chloral hydrate (1 ml, i.p.) and then perfused via the ascending aorta with 0.9% saline (100 ml) followed by 4% paraformaldehyde in 0.1 M borax buffer, pH 9.5, at 4°C (500-700 ml over 25 min). The brain was postfixed overnight at 4°C in the same fixative containing 10% sucrose. Coronal sections of the hypothalamus were obtained using a freezing microtome and five 1-in-5 series of 20 µm slices were collected on gelatin coated slides for analysis. One series was incubated with rabbit polyclonal anti-rat vasopressin antibodies (diluted 1:2000) or rabbit polyclonal anti-rat oxytocin antibodies (diluted 1:700; Insctar, Stillwater, MN, USA) for 48 h at 4°C [5]. The vasopressin antiserum was pre-adsorbed with oxytocin blocking peptide (Sigma Chemical Co., St. Louis, MO, USA) and the oxytocin antiserum was likewise preadsorved with vaso-

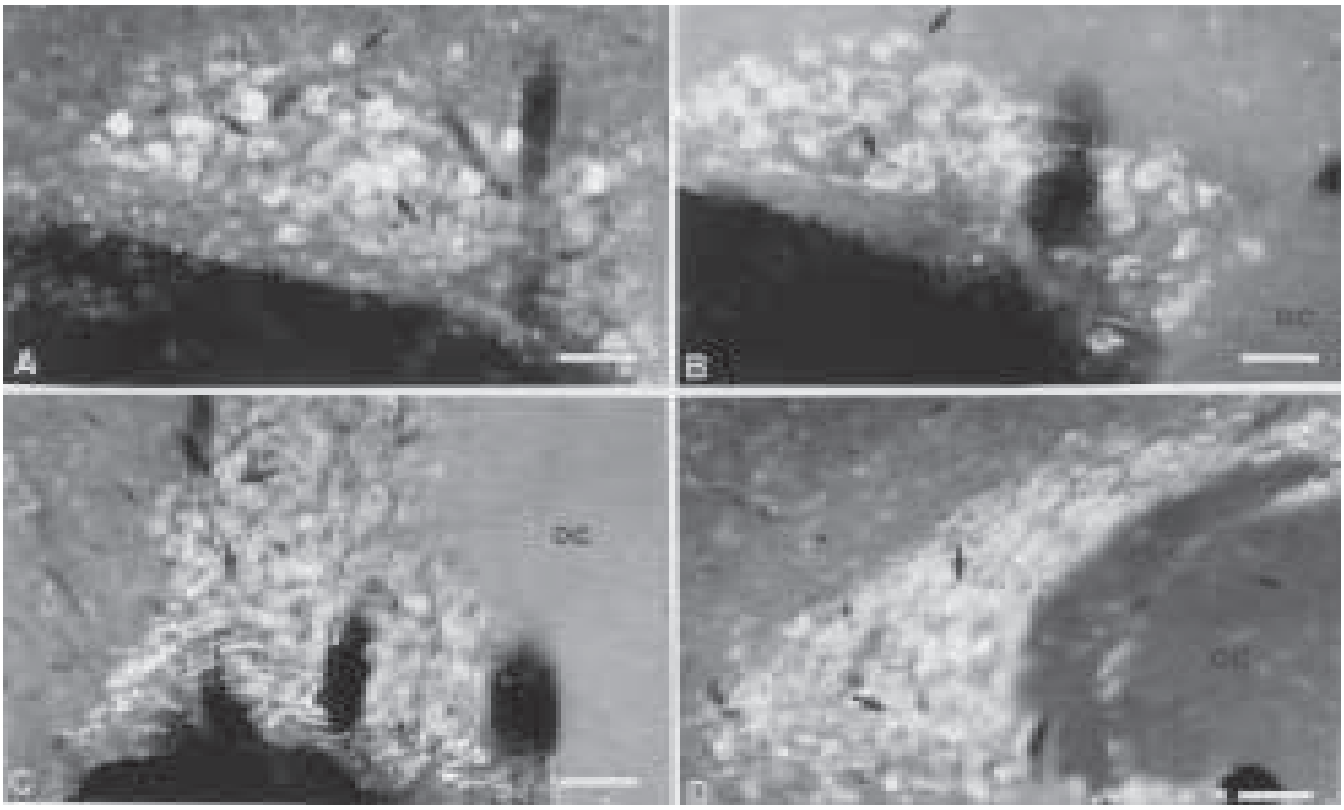

Figure 1. Immunofluorescence micrographs of oxytocin **(A, B)** and vasopressin **(C, D)** immunoreactive neurons (arrows) in the supraoptic nuclei of 3-month-old **(A, B)** and 15-month-old **(C, D)** rats. **(A, C),** control rats. **(B, D),** Colchicine-treated rats. The intensity of oxytocin and vasopressin immunoreactivity increased in the treated rats. oc, optic chiasm. Bars = 20 μm.

Table 1. Number of oxytocin (OX-IR) and vasopressin (VAS-IR) neurons in the central portion of the supraoptic nuclei.

	OX-IR		VAS-IR	
Age	**Control**	**Colchicine**	**Control**	**Colchicine**
3 months	31.8 ± 6.4 (n=6)	22.8 ± 3.9** (n=6)	36.8 ± 6.7* (n=6)	29.5 ± 6.8 (n=6)
15 months	35.0 ± 1.0 (n=4)	23.7 ± 2.8** (n=4)	25.7 ± 3.3* (n=4)	27.0 ± 1.4 (n=4)

The results are expressed as the mean ± S.E.M. n=number of rats used. *P=0.01, **P=0.001

pressin blocking peptide (Sigma) to reduce the possibility of cross-reactivity [36]. The slides were subsequently incubated with affinity-purified, fluorescein-conjugated goat anti-rabbit IgG (diluted 1:200; Tago Biosources Inc., Burlingame, CA, USA) for 1 h at room temperature. The sections were dried at room temperature and mounted under a coverslip in buffered glycerol (pH 8.5).

The labeled cells of the central portion of the supraoptic nuclei were counted using a Leica Epifluorescence microscope with a blue filter (BP 450 – 490 nm). The effects of age and of colchicine treatment on the distribution and fluorescence of OX-IR and VAS-IR neurons were compared statistically using two-factor analysis of variance (ANOVA) followed by multiple comparisons with the Tukey test. A P<0.05 value indicated significance. The results were expressed as the mean ± S.E.M. of the number of cells.

RESULTS

OX-IR neurons were observed in the dorsal and lateral parts of the supraoptic nuclei, with some cells also in the ventral region; VAS-IR neurons were observed in the ventral portion of the supraoptic nuclei. The intensity of the OX-IR and VAS-IR in the neurons in the supraoptic nucleus increased in colchicine-treated rats, irrespective of age (Fig.1). There were no significant differences in the number of OX-IR neurons among rats of the two age groups (Fig. 2, Table 1). However, the number of VAS-IR neurons decreased by 30.1% in 15-month-old rats compared to the

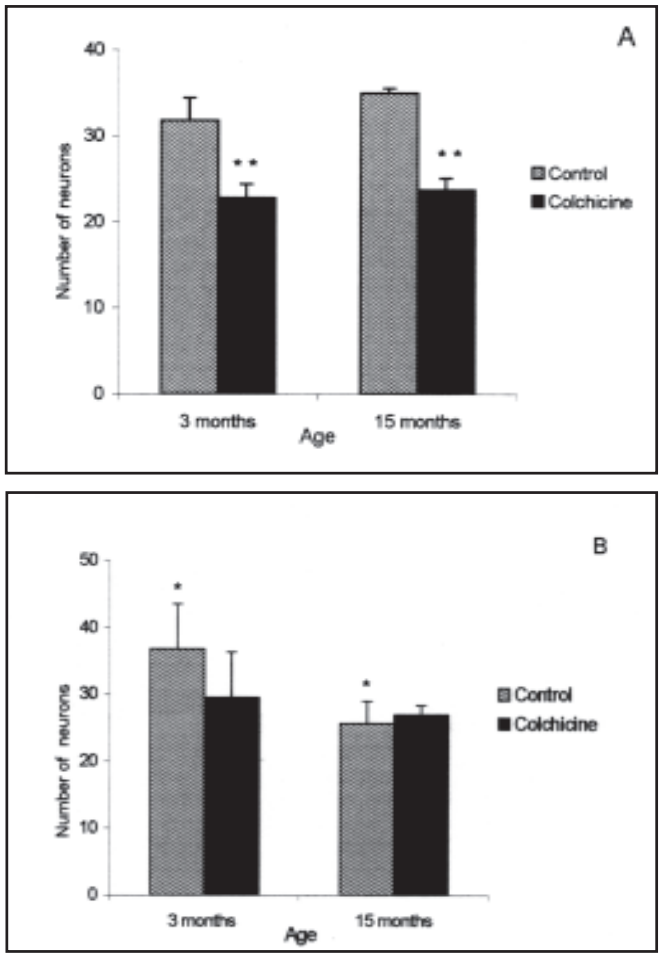

Figure 2. The effects of colchicine on the number of oxytocin **(A)** and vasopressin **(B)** immunoreactive neurons in the supraoptic nuclei of 3-month-old and 15-month-old rats. **P*=0.01 compared to controls at 3 months **(B)**, ***P*=0.001 compared to the controls **(A)**. The columns and bars represent the mean±SEM of the number of neurons in the central portion of the supraoptic nuclei.

younger rats (*P*=0.01) (Fig. 2, Table 1). The number of OX-IR neurons decreased significantly by 9.5% in colchicine-treated 3-month-old rats and by 11.1% in colchicine-treated 15-month-old rats (*P*=0.001). There was no significant difference in the number of VAS-IR neurons in untreated and colchicine-treated 3- and 15-month-old rats (Fig. 2, Table 1). Colchicine thus produced a similar effect in both age groups.

DISCUSSION

The organization of and relationship between oxytocin- and vasopressin-containing neurons have been extensively investigated [3,9,26,31,32,35]. Oxytocin-containing neurons are located mainly in the dorsal part of the supraoptic nuclei, while vasopressin-containing neurons are found predominantly in the ventral portion in untreated and colchicine-treated rats, irrespective of age. The central portion of the supraoptic nucleus was chosen for this study because it contains about 80% of the cells in this nucleus [19].

The adsorption technique used in this work allowed a better evaluation of the antigen because it decreased non-specific binding [25]. This was especially important because vasopressin and oxytocin have similar epitopes [35,36,39].

Colchicine administration resulted in similar intensities of staining OX-IR and VAS-IR neurons of the supraoptic nuclei. The intensity of the immunocytochemical reaction was stronger in colchicine-treated rats than in the controls, in agreement with previous reports [1,2,17,24,27,28]. Liu *et al.* [20] observed that colchicine increased OX-IR and VAS-IR levels in the supraoptic nuclei 48 h after injection. However, we observed a differential effect of colchicine on OX-IR and VAS-IR neurons in the supraoptic nuclei. The decrease in the number of OX-IR neurons in colchicine-treated rats was not seen with VAS-IR. Using another approach, Parish and Pickering [23] reported differential effects of colchicine on the transport of oxytocin and vasopressin in neurons of the hypothalamic-neurohypophysial system of rats, with oxytocin-containing neurons recovering more rapidly than vasopressin-containing ones. The dose of colchicine used in the present work was high and enough to destroy the micro-tubules and interrupt the axoplasmic flow [11,16,22,24]. Other studies have reported toxic effects that are longer lasting or irreversible. Goldschmidt and Steward [14] observed that colchicine injected into the dentate gyrus of the hippocampus in adult rats preferentially destroyed the dentate granule cells. Steward *et al*. [34] concluded that colchicine leads to the death of selected populations of neurons in different regions of the brain. The neurotoxicity of colchicine seems to be related to the destruction of microtubules, with some cells being selectively vulnerable to the disruption of microtubule-mediated transport [8,14]. Emerich and Walsh [10] observed that colchicine injection into the lateral cerebral ventricles exerted a direct toxic effect on cholinergic neurons and/or nerve terminals that resulted in the death of these neurons. The results of Barone *et al.* [4] indicate that nerve growth factor can modify colchicine-induced compensatory changes in hippocampal signal transduction and have transitory influences on cholinergic cells in the medial septum.

Colchicine binds to and depolymerizes microtubules [43]. A disruption of the cytoskeleton would have a profound impact on the structure of the neurons and on axonal transport, as well as on growth, development and regeneration following damage. Colchicine-induced destruction of cytoskeletal functions would likely affect the retrograde transport of trophic factors to oxytocinergic neurons and could contribute to the partial cell loss observed in this

work. The above factors may be associated with the decrease in the immunoreactivity and number of OX-IR neurons in the supraoptic nucleus. The mechanisms responsible for the differential effect of colchicine on OX-IR and VAS-IR neurons in the supraoptic nuclei are unknown. Further experiments are necessary to define the specific routes affected by this drug.

The effect of colchicine on OX-IR and VAS-IR neurons was similar in the two age groups studied, with a reduction in the number of OX-IR neurons. Aging differentially affected OX-IR and VAS-IR neurons. In 15-month-old untreated rats, the number of VAS-IR neurons was lower than in 3-month-old rats. In contrast, there was no difference in the number of OX-IR neurons in the two age groups. These data suggest a decreased function of neurosecretory vasopressin cells with senescence [12,13,40]. Data from another groups [29,30,42] suggest that VAS-IR declines with age in the hypothalamic-neurohypophysial system of monkeys and rats. Madeira *et al*. [21] observed that although there was an increase in size with age in both populations of supraotic nuclei, these changes were more marked in VAS-IR than OX-IR neurons. While there is general agreement that VAS-IR decreases with age, this decrease provides no information about the level of vasopressin neurosecretory activity. This decreased immunoreactivity VAS-IR declinescould reflect decreased peptide synthesis, increased transport, or increased release.

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