Differential Effects of Colchicine on Central Dopaminergic Neuronal Activity and Prolactin Secretion in Estrogen-Primed Ovariectomized Rats

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Abstract

Colchicine is a potent chemical that disrupts the assembly of microtubulin and affects the integrity of cytoskeleton. It is commonly used to block the axonal transport in neurons. Central administration of colchicine (48 μg/3 μl/rat) two days earlier significantly lowered 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the striatum and nucleus accumbens, both in the morning and in the afternoon. Median eminence DOPAC levels exhibit a diurnal change between morning and afternoon as previously shown. Colchicine treatment lowered and elevated median eminence DOPAC levels in the morning and afternoon, respectively. The estrogen-induced prolactin surge was also blocked. The findings indicate that neuronal inputs are necessary for maintaining basal activities in all dopaminergic neurons, while an inhibitory one predominates in the afternoon for TIDA neurons.

Key Words: tuberoinfundibular dopaminergic neuron, prolactin, median eminence, nucleus accumbens, striatum, circadian rhythm, female rat

Introduction

The anatomical distribution and physiological roles of central dopaminergic (DA) systems have been known for years (8). Neurons of nigrostriatal (NS) and mesolimbic (ML) DA systems originate from substantia nigra and ventral tegmental area in the midbrain and project to striatum (ST) and nucleus accumbens (NA), respectively. In contrast, the tuberoinfundibular (TI) DA neurons locate in the hypothalamic arcuate nucleus (ARN) and terminate in the median eminence (ME) (7). While the NSDA and MLDA systems are involved in the control of locomotor activity and rewarding circuit, the TIDA system is involved in neuroendocrine control of pituitary hormones (8). Dopamine released in the ME is carried to the anterior pituitary and acts as the prolactin (PRL)-inhibiting hormone (1, 10). Various neuroactive agents, e.g., opioids, GABA, cholinergic agonists, etc. can affect TIDA, MLDA, and NSDA neurons. Both similar and opposite effects have been observed (2, 7, 10).

Colchicine is a chemical that disrupts the assembly of microtubulin and destroys the integrity of cytoskeleton; both of which are indispensable for mitosis and cellular transport (11). Neuroscientists have used it as a neurotoxin to disrupt neuronal activities, especially the ones that involve axonal transport. In this study, we used intracerebroventricular (icv) injection of colchicine to determine its possible effect on activities of NSDA, MLDA and TIDA neurons, especially the diurnal changes observed in TIDA neurons and concomitant serum PRL levels.

Materials and Methods

Animals and Treatments

Adult female Sprague-Dawley rats, weighing between 200 and 250g, were purchased from National

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Yang-Ming University Animal Center (Taipei, Taiwan) and habituated for one week before used. All animals were housed in a temperature (23±1°C)- and light (light on between 0600 and 2000h)-controlled room with free access to rat chow and tap water. All rats were ovariectomized, and 1 week later (as day 0), implanted with subcutaneous silicone capsules (A-M Systems, Everett, WA; o.d., 3.18 mm; i.d., 1.57 mm; active length, 20 mm) containing esteradiol-17β (E_2; Sigma Chemical Co.; St. Louis, MO; 150 μg/ml corn oil; Sigma). Half of the rats received icv injection of colchicine (48 μg/3 μl/rat) using a stereotaxic instrument in the morning and the other half received aCSF (3 μl/rat) on day 4. All rats were decapitated on day 6 either at 1000 h in the morning or at 1500 or 1700h in the afternoon.

After decapitation, the brains were quickly removed and frozen on dry ice; serum samples were collected and stored at -20°C until assayed for PRL levels. The frozen brain was cryosectioned on the same day using a tabletop freezing microtome. Thick (600 μm), coronal sections were prepared and thaw mounted onto glass slides. The ME, NA and ST of each rat were dissected out from the brain sections using the micropunch technique (9). The punched out tissues were individually stored frozen in 40 μl of 0.15 M sodium phosphate buffer containing 0.65 mM sodium octanesulfonate, 0.5 mM EDTA, and 12% methanol, pH 2.6, until assayed.

Assays

The activities of central DA neurons were assessed by measuring the concentration of DOPAC using high performance liquid chromatography (HPLC) coupled with electrochemical detection (ECD) as previously reported (3, 4, 6, 12-15). DOPAC is the major metabolite of released dopamine in rat's brain by monoamine oxidase. Since the majority of released dopamine is taken back to presynaptic terminals and metabolized to DOPAC, the DOPAC concentration has been shown to be a reliable index for DA neuron activity (6, 8).

In brief, brain samples were thawed, sonicated and centrifuged. The supernatant was injected into an HPLC-ECD system (BAS LC480, with PM-80 pump, Rheodyne 7125 injector, phase II ODS column, 3.2 × 100 mm with 3 μm sphere, and LC-4C EC detector, Bioanalytical Systems Inc., West Lafayette, IN). The mobile phase was the same as the tissue buffer used in storing the punched brain tissues. The flow rate of the pump was 0.8 ml/min, and the oxidizing potential was set at +0.75 V. The tissue pellets were dissolved in 1.0 N NaOH and assayed for their protein contents (5). Data were expressed as nanograms DOPAC per milligram protein.

Serum PRL concentration was measured by RIA using the rat PRL (rPRL) RIA kit provided by Dr. A.V. Parlow of the National Hormone and Pituitary Program of the NIDDK, USA. The iodinated PRL was rPRL I-6, the PRL standard was rPRL RP-3, and the antibody was rPRL S-9. The sensitivity of the assay was lower than 50 pg/tube. The intra- and interassay coefficients of variance were 4% and 7% (n=20), respectively.

Statistical Analysis

One-way ANOVA followed by Student-Newman-Keuls' multiple-range test was used to test the significance of difference among groups. A $P< 0.05$ was considered as significant in difference.

Results

Effect of Colchicine on TIDA, NA and ST

A significant diurnal change of DOPAC levels in the ME was observed ($P< 0.01$; Fig. 1, upper panel) as previously reported (3, 4, 6, 12-15). Although treatment of colchicine for two days significantly lowered the morning DOPAC level in the ME ($P< 0.05$; Fig. 1, upper panel), it significantly increased its afternoon level ($P<0.01$; Fig. 1, upper panel).

No significant difference between the morning and afternoon DOPAC levels was observed in the ST and NA (Fig. 1, middle and lower panel). Treatment of colchicine significantly lowered both morning and afternoon DOPAC levels in the ST and NA ($P<0.01$; Fig. 1, middle and lower panel). No diurnal difference was observed in the effect of colchicine either.

Effect of Colchicine on Serum PRL Level

A significant afternoon PRL surge was observed in OVX+E_2 rats ($P<0.01$; Fig. 2, lower panel), also as reported (3, 4, 6, 12-15). While treatment of colchicine had no significant effect on PRL levels in the morning, it significantly blocked the afternoon PRL surge ($P <0.01$, Fig. 2, lower panel). The whole experiment was performed twice and the findings were confirmed. Since the first study only took the 1000 and 1500 h, but without the 1700 h samples, only the results of the second study were shown here. For the purpose of better comparison with ST and NA DOPAC, as well as with serum PRL levels, the data of ME DOPAC (Fig. 1, upper panel) are also presented in Fig. 2 (upper panel).

Discussion

Using colchicine as a tool, we obtained the

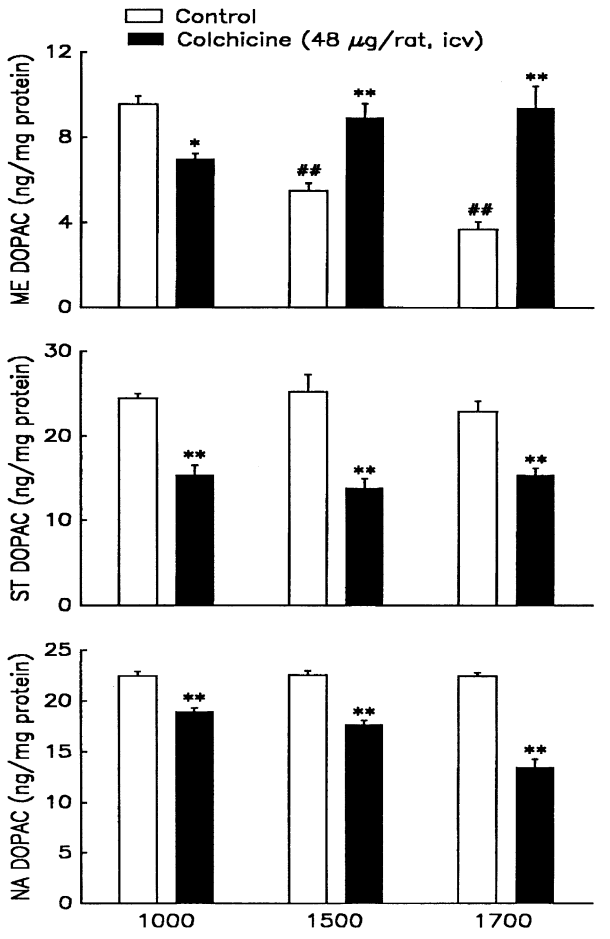

Fig. 1. Different effect of colchicine on ME, ST and NA DOPAC levels of OVX+E_2 rats. Colchicine (48 μg/3 μl/rat, icv) or saline was given around 0900 h and the rats were decapitated 2 days later. The vertical line above each bar represents the SEM (n=6). *, P<0.05; **, P<0.01 compared with the saline control at the same time points; ##, P<0.01 compared with the saline control at 1000 h.

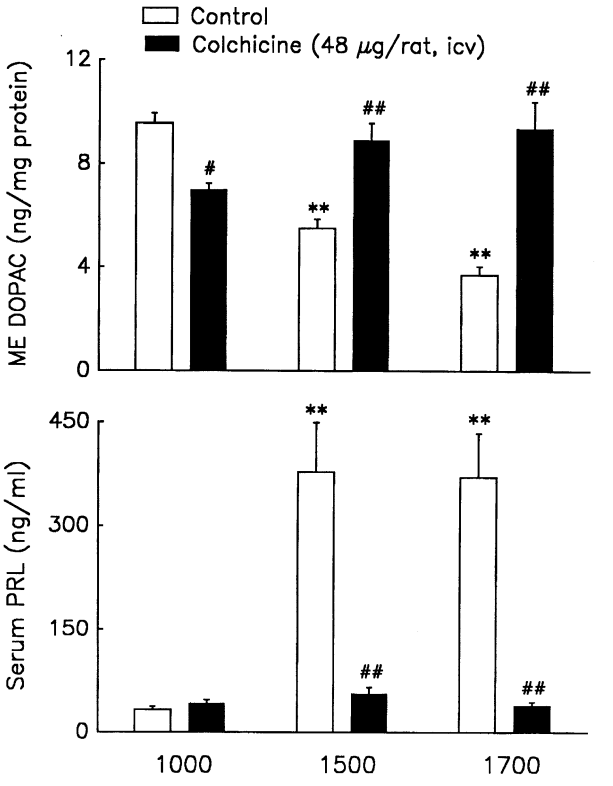

Fig. 2. Differential effect of colchicine on ME DOPAC and serum PRL levels in the morning and afternoon of OVX+E_2 rats. The vertical line above each bar represents the SEM (n=6). ##, P< 0.01 compared with the saline control at the same time points; **, P<0.01 compared with the saline control at 1000 h. Refer to Fig. 1 for further detail.

following findings in this study: 1) colchicine decreased MLDA and NSDA neuronal activities both in the morning and in the afternoon. 2) Colchicine exhibited differential effects on TIDA neuronal activities, i.e., lowering those in the morning and elevating those in the afternoon. 3) Colchicine blocked the afternoon PRL surge in OVX+E_2 rats.

The underlying mechanism of the diurnal change in TIDA neuronal activity has been extensively studied. Increased stimulatory input to TIDA neurons in the morning that diminishes in the afternoon and/or increased inhibitory input during the afternoon have been shown. For instance, an endogenous cholinergic tone, low in the morning and high in the afternoon, has been reported (12, 13), which may act via endogenous opioidergic system to inhibit the TIDA neurons (14). In addition, serotonin (4), GABA (3) and nitric oxide (15), have all been shown to play a role in lowering the TIDA neuronal activity in the afternoon.

Since colchicine blocks the axonal transport, thus neuronal inputs projecting to the DA neurons as well as the activities of DA neurons themselves should all be affected. The finding that the activities of both NSDA and MLDA neurons were lowered by colchicine treatment confirmed this notion. There was no diurnal difference in their responses either: both morning and afternoon DOPAC levels in the ST and NA were similarly affected.

As for the TIDA neurons, they not only exhibited diurnal changes as previously reported, their responses to colchicine also differed between morning and afternoon. In the morning when the activity is high, treatment of colchicine lowered it as it did to NSDA and MLDA neurons. In the afternoon when the activity is low, however, colchicine elevated it. It confirmed our belief that during the afternoon, the major input to the TIDA neurons should be inhibitory. Removing that inhibition by colchicine may have revealed the basal activity of TIDA neurons which was similar to their morning levels.

As for the estrogen-induced afternoon PRL surge, it has been repeatedly shown that once the diurnal rhythm of TIDA neurons is disrupted, the PRL surge is also affected. The TIDA rhythm has been eliminated by lesion of the suprachiasmatic nucleus (SCN) (6), and treatments of cholinergic or opioidergic antagonists (12, 14), nitric oxide synthase inhibitor (15), or serotonergic antagonist (4), etc. The present finding showing that colchicine prevented both rhythmic changes of TIDA neuronal activity and PRL secretion further strengthens that notion.

The TIDA neurons possess several characteristics that differ from other midbrain DA neurons (7, 10). Among them are sex specificity, circadian nature, and under the control of various humoral and neuronal factors. We have shown that lesion of the SCN, the major rhythm-generating center in the CNS, eliminates the diurnal rhythm of TIDA neuron (6). Thus, signal(s) originating from the SCN that reaches the TIDA neurons may be responsible for the generation of the TIDA rhythm, and which may be the one that was disrupted by colchicine treatment. As for the exact nature of the signal awaits further studies.

In summary, this is the first report that demonstrated differential effects of colchicine on central DA systems, and the importance of axonal transport for the occurrence of diurnal TIDA rhythm and serum PRL surge in OVX+E_2 rat.

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