Colchicine may interfere with the axonal transport of noradrenaline in the central noradrenergic neurons

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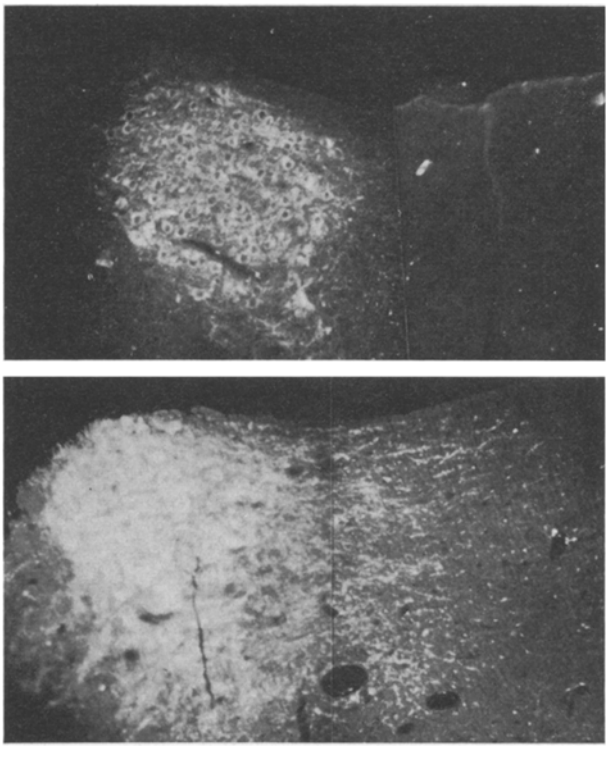
Summary. The administration of colchicine (50 μg) into the lateral ventricles induced the marked accumulation of noradrenaline in the locus coeruleus of rat. Lumicolchicine administration or stress loading failed to alter the noradrenaline levels, thus suggesting that this accumulation is related to the blockade of axonal flow in the central noradrenergic neurons.

The proximo-distal transport of noradrenaline (NA) is well demonstrated in the peripheral adrenergic neurons[3,4] (see also review by Dahlström[5] and Heslop[6]). The evidence for the involvement of microtubules in this transport is the widely observed inhibition of transport by the local application of antimitotic agents which disrupt microtubules and bind to the protein subunit of microtubules[7–9]. Although catecholamines in the central nervous system are also known to be transported from the cell bodies to the terminals[10,11], little is known whether this transport also involves microtubules. We have therefore examined this possibility by injecting colchicine, one of antimitotic agents, into the lateral ventricles of rats and measuring NA levels biochemically and histochemically in the locus coeruleus (LC), where the dorsal NA bundle innervating the cerebral cortex exclusively[12]. The LC was chosen because its location is close to the floor of the 4th ventricle and thus colchicine administered intraventricularly may easily be accessible to this nucleus. Our results showed that NA levels in the LC increased markedly after colchicine, but not after lumicolchicine administration.

Material and methods. Male Wistar rats weighing 200–300 g were anesthetized with ether and received injections of 20 μl (50 μg) of colchicine dissolved in physiological saline into the lateral ventricles. Animals often showed paraparesis and hematuria after 24–48 h and survived for a maximum of 4 days. Preliminary experiments showed that the injections of physiological saline alone had no influence on the levels of catecholamines in the LC, and therefore untreated animals served as controls in all experiments. At various intervals after the injection, the animals were lightly anesthetized with ether and killed by decapitation. The LC was dissected according to the procedure by Zigmond et al.[13] with a slight modification described previously[14]. Tissues were homogenized with 40 vol. of 0.1 N perchloric acid and centrifuged for 15 min at 1580 × g. The resulting supernatant were analyzed for catecholamines (NA plus adrenaline and dopamine) by the radiochemical method of Coyle and Henry[15]. In some experiments, NA alone was assayed by the method of Henry et al.[16]. NA contents measured by 2 methods coincided well, thus showing that the adrenaline levels are negligible in the LC. In other experiments, tissues were homogenized with 5 mM tris buffer (pH 7.4) containing 0.2% cutscum, and the tyrosine hydroxylase (TH) activity was measured by the $^{14}CO_2$ trapping method of Waymire et al.[17], which employed the partially purified hog kidney decarboxylase. Slight modifications were carried out in which tyrosine concentration was a 73 μM and a cofactor (6-methyl-tetrahydropterine) 1 mM.



Fluorescence micrographs of the locus coeruleus of rat. The cell bodies in LC have developed a much stronger fluorescence 2 days after colchicine treatment (below) than untreated (above) ones, so that the nuclei in the neurons have been masked. Also note that the treated axons can be more easily traced.

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Effects of colchicine, lumicolchicine administration and stress loading on NA levels in the LC

	NA (ng/region) Control	Experimental
Colchicine (50 μg)	18.0 ± 0.8(11)	29.9 ± 1.5(11)*
Lumicolchicine (400–500 μg)	18.2 ± 0.7(8)	17.8 ± 0.6(10)
Restraint (6h)	16.6 ± 0.7(8)	17.5 ± 0.7(8)

Rats were treated with colchicine or lumicolchicine into the lateral ventricles, or put in a wire-meshed cage and immersed in flowing water (15 °C) to the xyphoid process for 6 h. Rats were decapitated 2 days later. Results are expressed as means ± SE. Numbers of animals are shown in parenthesis. *Denotes the significant difference from controls at the level of 1%.

The tissues for catecholamine fluorescence microscopy were processed by the method of Falck and Hillarp[18]. The lumicolchicine was prepared from colchicine by the method of Wilson and Friedkin[19].

Results and discussion. NA contents in the LC increased markedly 2 days after colchicine administration (table). Dopamine (DA) contents also increased significantly from 2.0 ± 0.1 ng to 3.2 ± 0.2 ng (N = 4). The relatively low values of DA suggest that the region dissected as the LC had negligible contamination of dopaminergic neurons, and therefore the DA accumulated after colchicine is the one localized in LC as a precursor of NA. Consistent with the biochemical results, the histochemical study demonstrated the marked accumulation of fluorescence in the LC (figure). Not only the fluorescence in the cell bodies, but also that in the axons are more intense than that of control, so that the axons can be more easily recognized. To see the specificity of colchicine action, the effect of lumicolchicine was investigated, an isomer of colchicine, which has a much less microtubules binding capacity and is far less effective in blocking axonal transport in other neuronal systems[20,21]. Even 10 times higher doses of lumicolchicine than colchicine failed to alter the NA levels in the LC as well as the animal behavior (table). Since the intracisternal injection of colchicine induces marked behavior changes known as colchicine neuropathy in rabbits[22], we next investigated the possibility that this treatment resulted in a general stress, which in turn increased the activity of tyrosine hydroxylase[13], the rate limiting enzyme of NA synthesis, and thus increased the NA levels in the LC. However, the exposure of rats to stressful situations (restraint) failed to alter the NA levels despite increasing the tyrosine hydroxylase activity significantly from 1.97 ± 0.10 to 2.59 ± 0.16 nmoles DOPA formed/h region (N = 8 in each group). The morphological evidence available indicates that the intracisternal administration of some antimitotic agents induced a loss of neurotubules and a proliferation of filaments in the anterior horn cells in rabbits[22]. Also similar biochemical results to ours are available in which the intracisternal injection of colchicine markedly interrupted the rapid migration of labelled proteins in the hypoglossal and vagus nerve at the level of the perikaryon[23]. Our results, taken together with these observations, suggest that the blockade of axonal transport is responsible for the accumulation of NA in the LC as a consequence of the binding of colchicine to the neurotubules. However, a final conclusion is reserved until the fate of other intraaxonal constituents, such as catecholamine synthetizing enzymes, has been elucidated. A study along with this line is in progress in our laboratory.

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Dopamine beta-hydroxylase in human synovial fluid

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Summary. Dopamine beta-hydroxylase (DBH) activity has been found in synovial fluid obtained from human knees. The enzyme activity was about 5% of the activity found in the serum of the same control patients. DBH activity in synovial fluid of patients suffering osteoarthritis was 3 times higher.

Dopamine beta-hydroxylase (DBH), the enzyme that catalyzes the conversion of dopamine to noradrenaline (NA) in synaptic vesicles of sympathetic neurones, is released along with NA in response to physiological stimulation of adrenergic nerves[2]. Accumulation of DBH in the sera of animals and humans is now well established[3,4]. Also, the enzyme has been detected in lymph fluid of both dogs and cats, suggesting that after sympathetic stimulation the enzyme enters the blood in part through the lymph[5,6]. Since the protein contents of human synovial fluid appear to be identical with those of plasma[7], the possibility exists that DBH could also be present in synovial fluid. In this work, DBH activity was detected in synovial fluid of normal human knees and compared with the enzymic activity found in synovial fluid of pathological joints.

Material and methods. 19 patients (10 males and 7 females) suffering from nonjoint diseases were selected as controls. Ages ranged between 13 and 85 years. In addition, 12 patients (4 males and 8 females) suffering from osteoarthritis (degenerative joint disease) with ages ranging from 43 to 63 years were also studied. Of these, 10 patients had osteorarthritis of the knee and 2 other patients had osteoarthritis of the hip joint; 5 were in the initial stages of the disease and the remaining were in more advanced stages. Synovial fluid of the knee was taken under general anesthesia from control patients during non-osteoarthritic surgery, and from osteoarthritic patients during surgery of the knee or the hip joint.

Except for some patients with osteoarthritis in which the synovial fluid was taken by arthrotomy, fluid was always taken aseptically by paracentesis. Puncture was made