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

Dopamine beta-hydroxylase (DBH) activity in serum and synovial fluid of control and patients with osteoarthritis

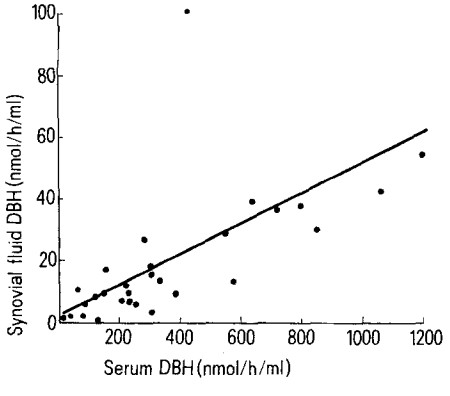
	N	Age (years)	Blood pressure (mm Hg) Systolic	Diastolic	DBH activity (nmol/h ml) Serum	Synovial fluid	S-DBH/SF-DBH
Control	17	46.6 ± 4.6 (13–85)	131 ± 5 (105–180)	74 ± 2.6 (60–90)	245.2 ± 42.2 (0–668)	10.9 ± 2.3 (0.4–36)	44.8 ± 18.2 (0–325)
Osteoarthritis	12	55.2 ± 2.1 (43–63)	147 ± 9.6 (90–190)	84 ± 5.2* (60–120)	467.7 ± 101.3* (41–1200)	30.3 ± 7.8** (2.4–102)	18.5 ± 3 (4.1–44)

DBH activity was assayed in 1 µl serum and 25 µl synovial fluid. The optimal pH for the first step of the reaction was 5; this step was run for 1 h in presence of 10 µmoles N-ethylmaleimide in order to overcome the effect of endogenous inhibitors. The adequate inactivation of enzyme inhibitors was further tested by adding to a duplicate of each sample a known amount of a partially purified bovine adrenal DBH. With these aliquots of serum and synovial fluid, the recoveries were always greater than 90%. The activity of a standard amount of partially purified bovine adrenal DBH decreased gradually when aliquots greater than 25 µl of synovial fluid were added. However, the enzyme activity was linear with 5–50 µl of synovial fluid. Data are means ± SE. N, number of patients. In parenthesis, range values. SD-BH = Serum DBH; SF-DBH = Synovial fluid DBH. * $p < 0.05$; ** $p < 0.01$, compared with controls.

in the outer border of the patello-femoral joint. Synovial fluid and blood obtained by venipuncture were immediately placed in ice-chilled tubes and centrifuged at 12,000 × g for 10 min. The supernatants from synovial fluid and serum were frozen at –20 °C until assayed for DBH activity. DBH activity was stable under these conditions for several weeks. Samples of synovial fluid contaminated with blood were discarded. Blood pressures were measured at the time of sampling. The method employed to assay DBH activity was slightly modified[8] from that described by Goldstein et al.[3].

Results. The table shows that synovial fluid of control patients contained a significant amount of DBH activity, but still only 5% the activity found in the serum of the same patients. The enzyme activity was easily measured in synovial fluid and, apparently, the endogenous inhibitors of the enzyme were much less potent when compared to those present in serum. Even though the range of variation of the values of DBH activity found in synovial fluid is large, it was still smaller than in serum. The table also shows individual serum to synovial fluid DBH ratios (S-DBH/SF-DBH). Excluding the 2 extreme values (0 and 325), it appears that ratios might be more useful in order to evaluate changes in synovial fluid DBH between individuals.

Patients suffering from degenerative joint disease had elevated serum and synovial fluid DBH activities. So, while serum DBH activity was almost twice as much as control, synovial fluid DBH increased 3 times. Therefore, S-DBH/SF-DBH decreased. Since DBH activity in human serum is age-dependent[9], we should compare these data only with control patients within the same range of ages. But even if patients younger than 43 years are excluded, the control group serum and synovial fluid DBH values remained similar.



The relationship between serum dopamine beta-hydroxylase (DBH) activity and synovial fluid DBH activity in humans. The correlation coefficient was 0.60; $p < 0.01$; N = 29.

Discussion. It is believed that the normal synovial membrane excludes plasma proteins of high molecular weight[10,11]. For example, fibrinogen, a protein of mol. wt about 300,000, is not found in normal synovial fluid; therefore it is unlikely that synovial fluid DBH, a protein of similar mol. wt[12], comes from the plasma. The figure shows a plot of serum DBH activity versus synovial fluid DBH activity which reveals that there is a poor but significant correlation between the enzymic activity of both compartments. When the serum and synovial fluid DBH activities were separately plotted for the control population and for the population consisting of osteoarthritic patients, correlation coefficients of 0.79 ($p < 0.01$) and 0.44 ($p > 0.05$) were obtained, respectively. Although poor, this positive correlation indicates that if DBH in the joint does not come from the plasma, at least there is some relationship between the DBH activities of both serum and synovial fluid. On the other hand, the ratio total plasma protein/total synovial fluid protein is of the order of 2.4 which is about 20 times lower than the ratio S-DBH/SF-DBH. Therefore, it has to be assumed that the synovial membrane must exclude the circulating DBH only par-

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tially, or that the enzyme found in the joint has a local source. It would be interesting to see if some other smaller adrenergic vesicular proteins, such as chromogranins which have a mol. wt of 70,000, and are also released along with NA during sympathetic nerve stimulation[2], are present in higher amounts than DBH in synovial fluid. This would clarify the source of the DBH present in synovial fluid. Little is known about the sympathetic innervation of the joint, but it may be that the enzyme comes from sympathetic nerves innervating the joint structures, such as the synovial membrane whose cells have secretory functions. These nerves might regulate the secretory functions of these cells, or other functions of the joints, such as the amount and composition of synovial fluid, a critical factor for the adequate lubrication of the joint cartilage.

Increased cardiac noradrenaline turnover in the rat after acute exposure to environmental heat

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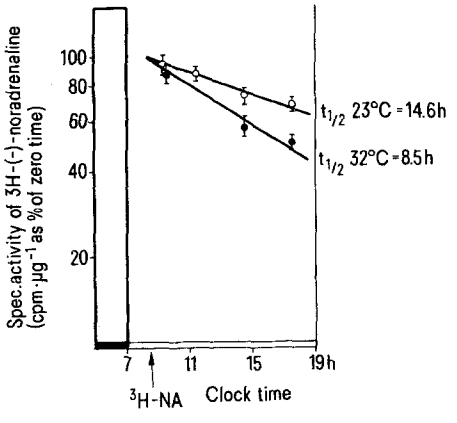
Summary. In male rats, the cardiac turnover of noradrenaline is significantly increased on acute exposure to an environmental temperature of 32 °C, when compared to control experiments performed at 23 °C.

Various environmental factors influence the metabolism and the turnover of noradrenaline in the rat heart, e.g. grouping of the animals[2], state of training[3], light and darkness[4], high altitude influence[5] and various factors which are stressing the animals[6]. Furthermore, acute and long-term exposure to cold increase the cardiac noradrenaline turnover in rats[7], and environmental heat increases the noradrenaline turnover in the hypothalamus[3], which plays an important role in the thermoregulation in the rat[8], and in other areas of the rat brain[9]. As the cardiac output and the heart rate was reported to be increased at an elevated room temperature[10], it was the aim of this study to investigate whether these changes may in turn be due to an increased turnover rate of the physiological transmitter substance, noradrenaline, in the rat heart.

Materials and methods. In the experiments, male Wistar rats (TNO W.70) of about 130–160 g were used. The animals were kept for at least 5 days under a controlled lighting schedule of 12 h light (7.00–19.00 h) alternating with 12 h darkness (19.00–7.00 h) with food and water ad libitum and at a room temperature of 23 ± 1 °C. The noradrenaline turnover in the hearts was determined in the period of light from the logarithmic decline of the specific activity after i.v. injection of 10 μCi/kg 3H-(–)-noradrenaline (Radiochemical Centre, Amersham, spec. activity 8.7 Ci/mmole) as described in detail previously[4]. The noradrenaline concentration was determined according to Chang[11]. Heat experiments were performed at a room temperature of 32 ± 1 °C, and in order to achieve this temperature the usual environment of the animals was heated up from 7.00 h until the beginning of the experiment at 8.30 h. Control experiments were performed in the same week at a normal room temperature of 23 ± 1 °C. Significance was tested by the unpaired 2-tailed Student's t-test, and linearity of the regression functions was proved by the F-distribution.



Decay of the specific activity of noradrenaline in the rat heart after i.v. injection of 10 μCi/kg 3 H-(–)-noradrenaline in the period of light at a room temperature of 23 °C (○—○) or 32 °C (●—●). Arrow indicates time of injection at 8.30 h (zero time). Each point represents the mean value ± SEM from 5 animals.

Results. The endogenous noradrenaline concentration in the hearts was increased by the acute exposure to heat from 0.94 ± 0.02 $\mu g \times g^{-1}$ (n = 43) at a normal temperature to 1.04 ± 0.02 $\mu g \times g^{-1}$ (n = 43). This increase was statistically significant ($p < 0.001$). In the figure, one representative experiment out of each experimental group is depicted, showing that the half-life of the specific activity of the cardiac noradrenaline is decreased when the environmental temperature is increased from 23 °C to 32 °C. All experimental results are summarized in the table. It can be seen that acute exposure to an elevated room temperature of 32 °C during light significantly increased the turnover rate of noradrenaline in the rat heart (0.069 $\mu g \times g^{-1} \times h^{-1}$) when compared with the control experiments performed at a room temperature of 23 °C (0.039 $\mu g \times g^{-1} \times h^{-1}$). Thus, the mean half-life was decreased to 10.2 h at 32 °C from 16.3 h at 23 °C.

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