Mean ± SE of enzyme activities measured in circular areas of the preparations, objective × 6 (indicator: intensity of staining expressed as optical density) in the cold-resistant LC3 and the parent L-As cells

Enzyme	Cells LC3 Mean ± SE	No. of fields	L-As Mean ± SE	No. of fields	Level of significance
Nonspecific esterase	90.85 ± 1.47	28	66.33 ± 0.65	25	0.1%
Acid phosphatase	94.82 ± 1.30	30	83.45 ± 1.39	30	0.1%
Adenosine triphosphatase	63.82 ± 0.16	40	59.20 ± 0.24	40	0.1%
Succinate dehydrogenase	79.80 ± 0.49	30	80.30 ± 0.76	30	NS
The same measured in 30 individual cells (objective × 40)					
Nonspecific esterase	146.86 ± 1.22		132.60 ± 0.85		0.001%
Acid phosphatase	130.70 ± 0.52		127.36 ± 0.53		0.001%
Adenosine triphosphatase	132.06 ± 0.96		123.20 ± 0.45		0.001%
Succinate dehydrogenase	139.50 ± 1.09		139.06 ± 1.44		NS

sues of cold-adapted animals in situ. These characteristics are not lost after many generations at 36 °C, and a comparison of some of their enzyme activities was therefore possible at this temperature where both cell types live undamaged.

The higher activity of ATPase and its localization on cell surfaces in the variant, expected in view of the better regulation of the intracellular K^+ concentration in the cold, confirmed changes in the constitution and function of the surfaces membranes detected also by direct potassium measurements[1], by scanning electron microscopy[9] and by microcinematography[10]. Further studies are necessary to distinguish precisely between the ATPase activity localized on cell surfaces and in the mitochondria. The increased activity of acid phosphatase as well as of other lysosomal enzymes[11] strengthens the hypothesis that lysosomes may be involved in cold adaptation[4]. The nonspecific esterase covers a wide field of reactions; esters of acyl-coenzyme A were suggested as important substrates for cellular respiration and thermogenesis[5], and our preliminary experiments showed also differences between the 2 cell types in the content of cholesterol esters. The lack of difference in the activity of succinate dehydrogenase found in repeated time-independent tests indicates either that this system is not activated at 36 °C in the LC3 cells, or that it is not involved in cold resistance.

Although it is not possible to see a direct analogy between the cold-adapted animal and the cold-resistant cell, yet such cells may offer a valuable model of some processes occuring in situ.

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Effects of denervation and local 6-hydroxydopamine injection on testicular growth in rats

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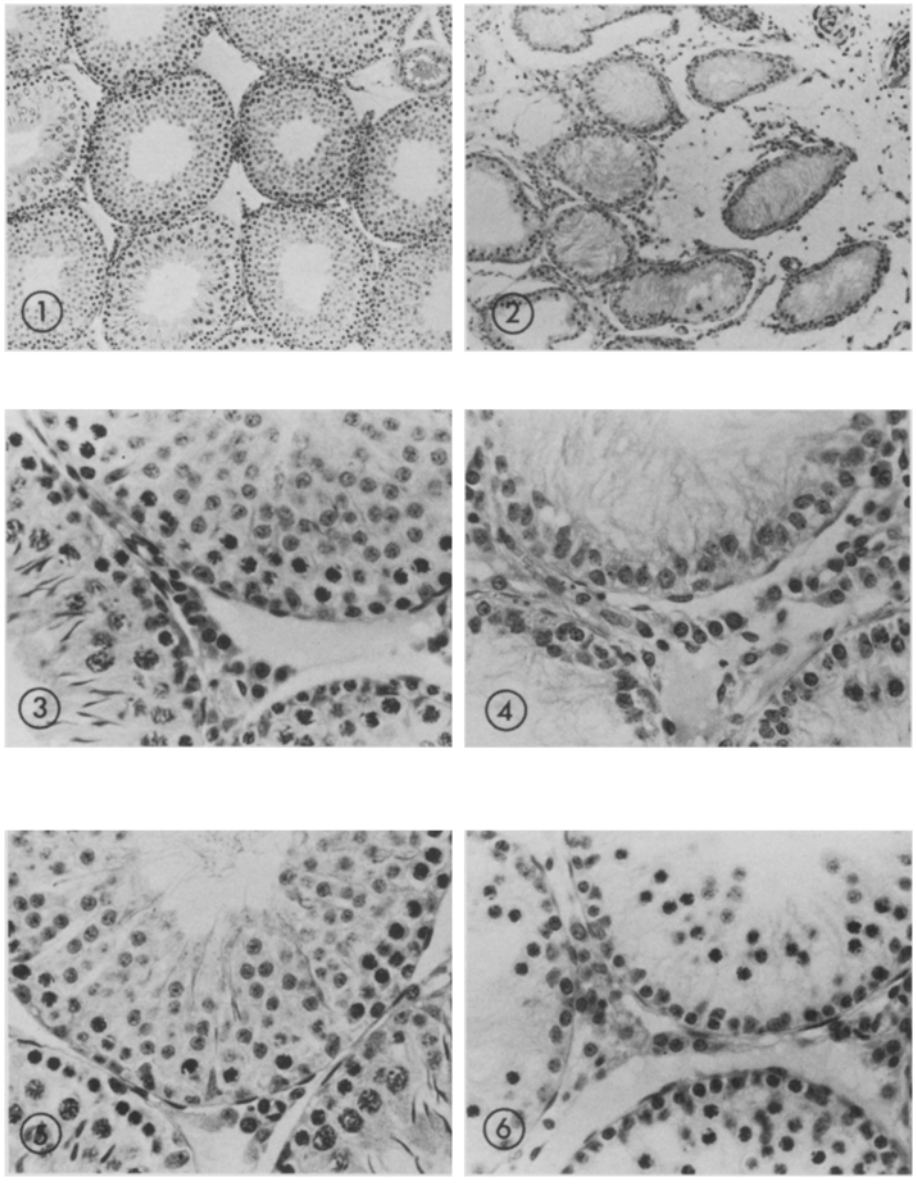
Summary. Denervation and local 6-hydroxydopamine injection on day 13 caused a decrease in the testicular weight by days 42 to 70, which was due primarily to a disturbance in the growth of seminiferous tubules.

Although many reports have documented the important role of the hypothalamo-hypophyseo-gonadal axis in testicular growth and function, little is known about the role of the nervous system. Kuntz[1] and Takahashi[2] reported degeneration and reduction of the germinal epithelium after removal of the sympathetic nerves innervating the testis in the dog and the guinea-pig, respectively. Other workers reported similar findings in the cat[3] and the rat[4], and the reduction of spermatogenesis after similar procedures in man[5–7]. Thus we examined the effects of denervation and local 6-hydroxydopamine (6OHDA) injection on the developmental growth of the testis in the rat, in order to elucidate the neural role.

Materials and methods. Male Wistar strain rats were anesthetized with ether, and 2-mm segments of the right superior and middle spermatic nerves were resected at a distance of 5 mm from the right testes at the age of 13, 19 or 21 days (days 13, 19 or 21; denervation experiments). 6-OHDA hydrobromide (16 µg/g b.wt) in 8 µl of 0.01% ascorbic acid solution was injected beneath the capsule of the right testis on days 13, 17, 21 and 26, whereas the same amount of ascorbic acid solution was injected into the left



Histology of the rat testis at 42 days of age. *1* Left(control) testis of a denervated rat (×77). *2* (treated) testis of a denervated rat (×77). *3* Left(control) testis of a denervated rat (×280). *4* Right (treated) testis of a denervated rat (×280). *5* Left(control) testis of a 6-OHDA-treated rat (×280). *6* Right(treated) testis of a 6-OHDA-treated rat (×280).

Effect of denervation and 6-hydroxydopamine injection on the testicular growth of rats

Group	Animal number	Age at operation (days)	Age at sacrifice (days)	Body weight (g)	Testicular weight Left (g)	Right (g)	Number and percentage of rats with right testis weight (operated) lower than 75% of that of the left one Number	(%)
A) Denervation								
A1	3	13	63	279± 6	1.69±0.01	0.12±0.02***	3	(100%)
A2	10	13	70	355± 7	1.74±0.06	0.25±0.13***	10	(100%)
A3	6	19	42	177± 1	0.78±0.05	0.38±0.16*	4	(67%)
A4	7	21	63	297±20	1.49±0.05	1.23±0.09	3	(43%)
B) 6-Hydroxydopamine injection								
B1	8	13	42	205± 6	0.98±0.02	0.35±0.06**	8	(100%)
B2	3	13	42	203± 7	0.98±0.05	0.47±0.07**	3	(100%)
B3	6	13	42	167± 4	0.84±0.02	0.54±0.04***	6	(100%)
B4	8	17	42	180± 5	0.97±0.02	0.77±0.02***	1	(13%)
B5	3	21	42	173±13	0.90±0.09	0.81±0.08	0	(0%)
B6	5	26	56	278± 7	1.44±0.03	1.26±0.08	0	(0%)
C) Normal								
C1	6	–	13	21.5±0.8	.026±0.001	.025±0.001	0	(0%)
C2	9	–	42	180± 3	0.73±0.03	0.75±0.04	0	(0%)
C3	4	–	42	200± 1	1.01±0.05	1.03±0.04	0	(0%)

Values are expressed by the mean± SEM. Significance: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

testis as a control (6-OHDA experiments). Animals were weaned on day 25, and were sacrificed between days 42 and 70: After the testes had been weighed, they were fixed with Bouin's solution, embedded in paraffin, sliced and stained with hematoxylin and eosin for histological examination. Statistical analysis was performed by the t-test.

Results and discussion. The table shows the testicular weights and the incidence of growth retardation. Denervation caused a significant reduction in the right testicular weight as compared to the left except in the group operated on the 21st day of life. The incidence of rats with a right-testis weight which was less than 75% of the left testicular weight decreased with increasing age at the time of operation. From the gradual reduction in the incidence from 100% (operation at day 13) to 43% (operation at day 21), it may be speculated that there might be a critical time period up to the age of 15 days for the nervous system in promoting testicular growth. After the rats are 15 days old, the neural effect may be overridden by hormonal activities, since gonadotropins and testosterone levels in blood dramatically increase during days 15–20 after birth[8,9]. 6-OHDA, which destroys sympathetic nerve terminals, was injected into the right testis. This local chemical sympathectomy retarted testicular growth in treated rats (B1–B6), and there was also a gradual reduction in the incidence at the 75% criteria level. No compensatory hypertrophy was observed in the untreated (left) testes up to at least 42 days after birth in the operated group (A3, B1–B5); i.e., the weights of the left testes of the operated rats did not differ from those in control animals. Photographs of the histological preparations of the denervated or 6-OHDA-treated testes are shown in the figure. Seminiferous epithelial cells of denervated testes were degenerated and reduced, some portions of the seminiferous tubules were destroyed, digested, absorbed and displaced by fibrous tissue, and mature sperm was not seen in any tubules. Similar findings were seen in the preparation from 6-OHDA-injected testes, although there was less degeneration than in the surgically denervated testes. The interstitial tissue including Leydig cells appeared to be intact in the right testes of the above 2 groups. These results indicate that the sympathetic nervous system is most probably important for testicular growth, especially of seminiferous tubules. Gerendai et al.[10] have recently suggested the importance of adrenergic neural elements in ovarian compensatory hypertrophy, using local 6-OHDA administration, and Bercu et al.[11] have suggested a critical period for hormonal effects on testicular growth in neonatal rats using LH-RH antiserum.

Whether the role of the nervous system is through a direct effect on the testicular cell, through androgen secretion from Leydig cells, through the control of blood flow, or through other factors is currently under investigation.

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Central influence of vasopressin on baroreceptor reflex in normotensive rats and its lack in spontaneously hypertensive rats (SHR)

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Summary. In normotensive Wistar rats intracerebroventricular (i.c.v.) injection of 30 mU of lysine[8]-vasopressin enhances significantly the cardiovagal reflex bradycardia induced by a blood pressure rise. This effect is absent in spontaneously hypertensive rats (SHR) of the Okamoto strain.

The pressor effect of vasopressin is very strongly buffered by the baroreceptor reflex[1–3]. An enhancement of the sensitivity to the pressor action of vasopressin follows baroreceptor denervation[3,4]. An increase in pressor response to vasopressin has been observed in SHR[5] and in New Zealand genetically hypertensive rats[6] as compared to normotensive rats. A decrease in cardiac output and heart rate in conscious dogs after vasopressin administration in a dose subthreshold for pressor effect has recently been observed. No such decrease occured after baroreceptor denervation, a finding suggesting that vasopressin facilitates the baroreflex response[4].

The present investigation was designed to determine the effects of a central application of vasopressin on the cardiovagal component of the baroreceptor reflex in SHR of Okamoto strain and in control normotensive rats of matched age.

Materials and methods. Eight 4-months old male SHR derived from the Okamoto strain and 9 normotensive male Wistar rats were selected at random and anesthetized with urethane (1.3 g/kg i.p.). The arterial blood pressure was measured from a catheter tied into the femoral artery using a Nicotron transducer. Heart rate and heart period were calculated from the ECG recording. I.v. administration of drugs was made by cannulation of the femoral vein. For intracerebroventricular (i.c.v.) injections rats were placed in a David Kopf stereotactic instrument and a trephine hole was drilled into the skull 1.0 mm lateral and 1.0 mm posterior to the bregma. 30 mU of lysine[8]-vasopressin was injected into the lateral ventricle in a 3 µl vol., with a cannula of 0.4 mm outer diameter introduced at a depth of 4–4.5 mm from the top of the skull. At the end of each experiment 3 µl of Evans blue dye was injected into the lateral ventricle, the animal was sacrificed, the brain sectioned and ventricular staining checked. To test the specificity of the observed central effect of vasopressin control experiments were carried out, in which a 3 µl vol. of artificial cerebrospinal fluid[7] was introduced into the lateral ventricle. No significant change