Ascorbic acid in brown adipose tissue: effect of cold acclimation and high intake of the vitamin[1]

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Summary. The ascorbic acid content of brown adipose tissue increased 62% when rats underwent cold acclimation; however, the increase was 126% when rats living in the cold were fed a basal diet supplemented with 2% ascorbic acid. These findings suggest a role for ascorbic acid in this tissue during nonshivering thermogenesis.

When a homeothermic animal is exposed to a cold environment, it produces heat by shivering in order to maintain a normal body temperature. If cold exposure persists for a long period, a different mechanism of heat production, usually referred to as nonshivering thermogenesis (NST), is utilized and the animal is described as cold-acclimated (CA). Recent work indicates that brown adipose tissue (BAT) is probably the major site of NST in the adult CA rat[3].

Early work indicated that ascorbic acid (AA) administration prolonged the survival time of animals exposed to cold stress[4]. During cold acclimation AA levels increased in kidneys, liver, testes, adrenal gland[5] and BAT[6]. In previous experiments carried out in this laboratory, rats at room temperature and fed a diet supplemented with AA did not increase their stores of the vitamin in BAT although significantly higher levels were found in serum, liver, kidney and superior cervical ganglia (SCG)[7].

The present paper concerns the presence of AA in BAT in rats undergoing cold acclimation and the effect of a high intake of the vitamin.

Materials and methods. 32 male Wistar rats (Canadian Breeding Farm, St. Constant, Québec) of 201.8 ± 1.0 g b.wt (mean ± SEM), were randomly separated into 4 equal groups. Group A: basal diet; room temperature (25–28 °C); group B: basal diet supplemented with 2% AA; room temperature; group C: basal diet; cold room (4–6 °C); group D: basal diet supplemented with 2% AA; cold room. Groups A and C were fed the basal diet alone[8] and groups B and D were fed the basal diet to which was added 20 g of L-ascorbic acid (ICN Pharmaceuticals, Inc.) per kg of diet in corn starch. Diets were prepared at 2-week intervals and stored at 5 °C. Feed and water were supplied fresh daily ad libitum. Groups A and B were kept in a room at 25–28 °C and were referred to as warm-acclimated (WA) rats. Groups C and D (CA rats) were kept in a cold chamber at 4–6 °C. Aniamls were kept in individual cages and were weighed weekly. After 5 weeks on the experiment, the rats were decapitated and tissues were immediately dissected, weighed and stored at −75 °C.

AA was determined in tissues by an automated method described previously[9]. Protein was measured by the method of Lowry et al.[10] with bovine serum albumin as the standard. Student's t-test was used to evaluate statistical significance[11].

Results and discussion. Body weights (g) at the time of decapitation were: A) 380.5 ± 7.0 (SEM); B) 382.5 ± 6.9; C) 360.9 ± 8.8; and D) 357.1 ± 8.8. As expected, the growth rates of the rats in the cold room (groups C and D) were slightly lower than those of corresponding controls at room temperature (groups A and B). The high intake of ascorbic acid had no significant effect on body weight. No significant differences were observed in the weights of superior cervical ganglia (SCG), adrenal glands and liver among the 4 different groups of rats.

A high intake of ascorbic acid had no effect on the weight, protein or ascorbic acid contents of interscapular brown adipose tissue (IBAT) in rats at room temperature (groups A and B). However, the known effects of cold acclimation upon tissue weight, protein and ascorbic acid content were confirmed by comparing groups A and C (table). Page and Babineau[6] reported an even greater increase in ascorbic acid concentration in IBAT due to the cold, but their rats remained in the cold room for a period of 16–18 weeks rather than 5 weeks as in the present experiment.

In the rats fed ascorbic acid in the cold (group D), the IBAT was lower in weight than that in rats in the cold fed the basal diet but the protein and ascorbic acid levels in the tissue were, in contrast, significantly higher. The lower tissue weight was probably due to a reduction in water or lipid content as indicated by the relative increase in protein content, and it did not necessarily indicate an impairment in the general growth of the tissue. Moreover, the rats in group D appeared to be as fully cold acclimated as those in group C.

As the sympathetic nervous system is directly involved in nonshivering thermogenesis[12], it was decided to study the ascorbic acid content in the superior cervical ganglia in the 4 groups of rats. Ascorbic acid levels (μg/pair of ganglia) were as follows: A) 2.03 ± 0.15 (SD); B) 2.32 ± 0.29; C) 2.08 ± 0.28; D) 2.42 ± 0.34. Surprisingly, no effect due to the cold could be observed comparing A vs C or B vs D. However, significant increases ($p < 0.05$) were observed by feeding the ascorbic acid supplemented diet to rats at room temperature or rats living in the cold room. These results confirm our previous observation that SCG stores more

Effect of diet and environmental temperature on IBAT* in the rat

	Tissue weight (g)	p	Protein content (mg/g tissue)	p	AA content (μg/g tissue)	p
Room temperature (WA)						
Group A (basal diet)	0.53 ± 0.07	A vs B	105.6 ± 11.7	A vs B	49.7 ± 12.0	A vs B
Group B (basal diet + AA)	0.44 ± 0.09	NS	111.6 ± 10.8	NS	50.9 ± 6.1	NS
Cold room (CA)						
Group C (basal diet)	1.25 ± 0.22	C vs D	128.6 ± 8.9	C vs D	80.3 ± 16.8	C vs D
Group D (basal diet + AA)	0.88 ± 0.15	< 0.005	144.8 ± 12.3	0.010	112.3 ± 21.2	< 0.005
p A vs C	< 0.001		< 0.001		< 0.001	
p B vs D	< 0.001		< 0.001		< 0.001	

The values represent the mean ± SD (8 rats in each group). Differences are regarded to be significant if p is 0.05 or less. * Interscapular brown adipose tissue.

ascorbic acid when large amounts of the vitamin are added to the diet[7].

These findings suggest that ascorbic acid has an active role in brown adipose tissue in rats undergoing cold acclimation. In this process the tissue undergoes morphological and biochemical changes which result in nonshivering thermogenesis[13].

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Enhanced depressor reflex by stimulation of superior cervical ganglion and by propranolol[1]

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Summary. The systemic depressor reflex in cats evoked by inflation of an intrasinusal balloon is enhanced by stimulation of the superior cervical ganglion and by close infusion of 20 µg/ml/min of (d, l)propranolol.

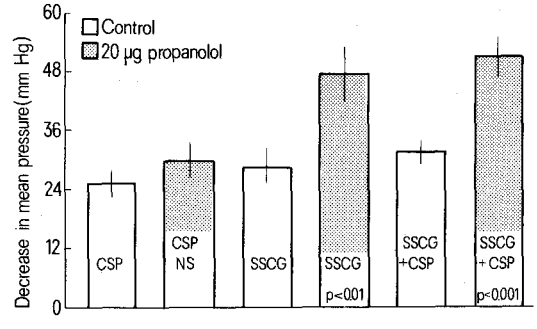
The functional role of the sympathetic nerves supplying the carotid sinus has been disputed[4], but a number of studies claim that stimulation of these nerves evokes a decrease in carotid sinus diameter[5], an increase in sinus nerve activity[6] and inconsistent depressor responses[7]. Recent studies also suggest that propranolol produces an increase in sinus nerve activity in the hydrodynamically restricted sinus, probably by decreasing sinus distensibility[8]. Constriction of smooth muscle by propranolol has been reported both in the guinea-pig trachea[8] and vascular bed of the rabbit ear[9]. Both tissues also receive a postganglionic sympathetic nerve supply from either the superior cervical (SCG) or stellate ganglia. This evidence might suggest that propranolol enhances smooth muscle tone through an interaction with this sympathetic innervation. The present investigation was designed to determine if the depressor reflex, in response to an increase in intrasinusal pressure, was enhanced by stimulation of the superior cervical ganglion (SSCG) and which modality - sinus pressure or stimulation - was most influenced by (d,l)propranolol.

Materials and methods. 7 cats weighing 2–3 kg were selected at random and anesthetized with pentobarbital sodium (50 mg/kg). Tracheal and venous (femoral vein) catheters were inserted. A femoral arterial cannual was connected to a pressure transducer and oscillographic paper recorder. One carotid sinus was isolated by typing the carotid artery above and below the sinus. All collateral circulation was excluded except the lingual artery which was cannulated with a 0.5 mm diameter catheter.

The ascending sympathetic trunk just caudal to the SCG was cut and led through and 'O' ring stimulating electrode. The latter was connected to a Grass stimulator and isolation unit. The ascending and descending vagus nerve was carefully excluded from the region of the electrode by microdissection and extirpation of at least 10 mm of nerve above and below the electrode. The sympathetic nerve was then bathed in warmed mineral oil and stimulated at 8–15 V and 8/sec, threshold was monitored via the nictitating membrane. A Berman No. SC301 angiographic balloon catheter was inserted into the carotid artery and advanced cranially and, by palpation, positioned within the bifurcation of the sinus. The catheter, consisting of the balloon tip and perforated collar for drug administration, was then inflated to a pressure of 180 mm Hg which displaced a volume of approximately 0.10 cm^3.

When a consistent depressor response to balloon inflation (CSP) had been established, the SCG was stimulated alone, and then in combination with balloon inflation. After repeating these 3 events a number of times, propranolol (20 µg/ml/min) was flushed through the sinus via the perforated neck of the catheter and out the sinus via the lingual artery. The tests were then repeated. In some cases, isoproterenol was infused before and after propranolol, but in no case did this catecholamine modify the responses obtained.

Results and discussion. Both CSP and SSCG reduced systemic blood pressure and in combination, they produced an enhanced reduction of this parameter. Propranolol intensified the depressor response evoked by SSCG. When combined, the depressor response was enhanced to an amount more nearly equal to that produced by SSCG alone. This is shown in the bar graph from data in 7 cats evaluated for statistical significance. As shown in the figure, SSCG was the modality most significantly effected by propranolol. This, in turn, contributed to the significant enhancement of



Statistical comparison in 7 cats of the effects of balloon inflation (CSP), sympathetic stimulation (SSCG) and combination (CSP+SSCG) on systemic pressure. Shaded bars and levels of significance (p values) represent change (± SE) to each modality of stimulation after exposure to d,l-propranolol.