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Norepinephrine Levels in Various Areas of Rat Brain during Cold Acclimation* (32624)

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Norepinephrine (NE) released from peripheral sympathetic nerve endings is believed to function as a mediator for nonshivering thermogenesis in animals subjected to prolonged cold (1), and in the newborn (2,3). NE also occurs in the brain, within neurons (4), where its concentration in the hypothalamus is relatively high (5). The hypothalamus is known to contain thermodectors and to play an important role in the control of body temperature (6). Based on work with cats and dogs, Feldberg and Myers (7) have suggested that the release of hypothalamic stores of both NE and 5-hydroxytryptamine (5-HT) exert a controlling influence over the regulation of body temperature. If release of these amines from hypothalamic sites is necessary for central regulation of body temperature, changes in their hypothalamic levels might be expected to occur following exposure to cold. In previous work we (8) found no changes in hypothalamic or whole brain levels of NE or 5-HT following exposure of rats to cold of 2°C for up to 24 hours. However, following more prolonged exposure of rats to cold of up to 30 days, whole brain NE levels were found to increase late in the acclimation period, while 5-HT levels remained unchanged throughout.

The purpose of the present study was to determine whether the increase in NE previously observed in whole brain during prolonged exposure to cold (8) occurs selectively in the hypothalamus, or whether it also occurs in other brain areas of relatively high amine content, such as the midbrain and medulla oblongata.

Materials and Methods. A total of 108 male, Sprague-Dawley strain rats, obtained from Charles River Breeding Laboratories, and weighing between 130 and 175 gm, were used in this study. Half the group was exposed to cold of 1°C (0.5–3°C) in a cold room having a relative humidity of 70%. They were kept two per cage in metal cages having wire-mesh floors and fronts and measuring 8 inches wide, 7 inches high and 10 inches deep. The remaining group was housed in similar cages, two per cage, in air-conditioned animal facilities kept at a constant temperature of 25°C, with a relative humidity of 60%. Food (Purina Rat Chow) and tap water were always available *ad libitum*.

At 1, 15, and 30 days following exposure to 1 and 25°C, the body temperatures of equal numbers of both groups of rats were obtained by means of a thermistor probe inserted 4 cm into the colon and connected to a telethermometer. These measurements were taken while the animals remained exposed to either the 1 or 25°C environment. The animals were then immediately decapitated, their brains were removed, and the various brain areas were quickly dissected out for extraction and determination of NE. The extraction method for NE was a modification of that of Vogt (9) and its chromatographic separation and determination on the blood pressure of the pithed rat was a modification of the method of Crawford and Outschoorn (10). The "hypothalamus" was removed from the brain by a circular cut following the Circle of Willis, 3–4 mm deep, extending partly into the thalamus, and contained the major hypothalamic nuclei, the optic chiasma (rostrally) and the mammillary bodies (caudally). The "midbrain" was removed by cuts midway across the pons and cerebral peduncles and included both superior and inferior colliculi. The "medulla"

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TABLE I. Norepinephrine Levels in Rat Brain during Cold Acclimation.

Days at 1 or 25°C	Norepinephrine levels (μg/gm) means ± SE								
	Hypothalamus			Midbrain			Medulla		
	1°C	25°C	p	1°C	25°C	p	1°C	25°C	p
1	1.28	1.55		.54	.58		.66	.61	
	±.08	±.21	.28	±.01	±.05	>.50	±.07	±.04	>.50
	(4)[a]	(4)		(4)	(4)		(4)	(4)	
15	1.15	.94		.57	.57		.56	.55	
	±.12	±.05	.18	±.04	±.05	>.50	±.07	±.03	>.50
	(4)	(4)		(4)	(4)		(4)	(4)	
30	1.70	1.30		.56	.44		.75	.62	
	±.42	±.05	.24	±.08	±.05	.22	±.05	±.06	.12
	(4)	(7)		(10)	(10)		(9)	(9)	

[a] Numbers in parentheses indicate number of determinations, 3 brains per determination.

included the area from the midpons to the level of C-1 of the spinal cord. The described areas from 3 brains were pooled for each extraction and determination. The amount of each extract obtained permitted two determinations on the rat blood pressure, the mean of the two values being that used in the results. The values thus obtained were added and a mean was obtained for each day at both ambient temperatures. NE levels are expressed as μg of amine base per gm of tissue (wet weight). To determine whether the prolonged cold-exposure influenced the extent of hydration of the brain, dry weights of samples of the various brain areas, following 30 days of cold exposure, were obtained by drying in an oven at 65°C for 3 days.

Student's t test was used to determine the statistical significance of the differences between means of the various experimental parameters. The probability values (p) of such differences occurring due to chance are reported in the "Results" section.

Results. The results of the NE determinations are shown in Table I. NE levels in the hypothalamus were slighty increased at 15 and 30 days, while in the medulla and midbrain, levels increased slightly only at 30 days. The timing of these increases, late in the acclimation period, and their magnitude, closely follow the pattern previously observed for whole brain (8). Although the increase in hypothalamic NE at 15 days seems to precede that of the medulla and midbrain at 30 days, the eventual increase seen in these latter areas demonstrates that the increases are not uniquely confined to the hypothalamus, but occur in other brain areas as well. The small decrease in hypothalamic NE observed following 1 day of cold was similar to that previously observed in the hypothalamus after 4 hours of cold (8). This small depletion is probably a nonspecific stress effect since it has also been observed to occur in brain following other types of stress (11).

The colonic temperatures of these animals, obtained just before killing, are shown in Table II. The greatest difference in mean colonic temperatures between cold-exposed and room temperature rats occurred at day 1, when there was a mean hypothermia of 0.8°C in the cold-exposed animals. The magnitude of this hypothermia diminishes with increasing length of cold-exposure, and these results are also similar to those previously obtained (8). There are no apparent relationships between the colonic temperatures of the animals and their brain NE levels.

The mean body weights of the two groups of rats before and during cold-exposure are given in Table II. The cold-exposed rats gain less weight than those at room temperature, presumably because the demand for metabolic energy in the cold-exposed animals prevents the storage of as much fat as those at room temperature. It has been reported that rat brain NE levels increase with increasing age and weight (12). Since the cold-exposed ani-

TABLE II. Colonic Temperatures of Rats during Cold Acclimation.

Days at 1 or 25°C	Colonic temperatures (°C) means ± SE		
	1°C	25°C	p
1	36.1 ± .11 (12)[a]	36.9 ± .08 (12)	<.001
15	36.9 ± .14 (12)	36.9 ± .07 (12)	>.50
30	36.9 ± .09 (30)	37.2 ± .06 (30)	<.01

[a] Numbers in parentheses indicate number of animals.

TABLE III. Body Weights of Rats during Cold Acclimation.

Days at 1 or 25°C	Body weights (gm) means ± SE		
	1°C	25°C	p
0	160.8 ± 1.2 (54)[a]	159.6 ± 2.0 (54)	>.50
1	160.5 ± 2.4 (12)	166.9 ± 2.9 (12)	.09
15	213.6 ± 5.1 (12)	252.1 ± 3.1 (12)	<.001
30	306.8 ± 5.1 (29)	339.8 ± 5.3 (30)	<.001

[a] Numbers in parentheses indicate number of animals.

mals weighed less than those at room temperature, their brain NE levels might also have been expected to be slightly less. Since they were, in fact, increased, the differences in amine levels cannot be related to the differences in body weights.

As is indicated in Table IV, there were no appreciable differences in the dry weights of the various brain subareas between 30-day cold-exposed and room temperature groups. The differences in NE levels cannot, therefore, be related to differences in brain hydration.

Discussion. Leduc (13) and others have shown that NE and other catecholamines increase in various peripheral organs, such as adrenal gland, heart, spleen, liver, and skeletal muscle, following cold-exposure. These increases conceivably occur in the sympathetic nerves innervating these organs and represent an increased demand for NE as the mediator for nonshivering thermogenesis. Since the magnitude and time course for the increase of NE in the various brain areas during the present study was similar to that previously found in whole brain (8) and other tissues (13) it is likely that these increases are all derived from a common stimulus to metabolism. The generalized nature of the increase throughout the brain would seem to argue against a unique role for hypothalamic NE in the acclimation process.

Although much of the NE in brain undoubtedly exists in the neurons of the central nervous system (4), some portion of the total NE may be associated with the vasomotor nerves accompanying the cerebral blood vessels. Since the observed increases in brain NE were small, relative to the total amount in brain, and do not seem to be restricted to circumscribed brain areas commonly associated with temperature regulation, it is possible that these increases occur in sympathetic vasomotor nerves rather than at synaptic terminals of central neurons. If such were the case, the observed increases in NE may be of little significance in the neurohumoral events involved in the regulation of body temperature by the central nervous system. The present studies give no indication as to whether the NE increases occur in vasomotor nerves, central neurons, or both. Even if it were established that these increases occur within central neurons, the physiological significance of these increases would remain questionable, as there appears to be some controversy as to the functional significance of

TABLE IV. Dry Weights of Rat Brain Areas Following 30 Days at 1 or 25°C.

Brain area	Solids (%)[a]	
	1°C	25°C
Whole brain (minus cerebellum)	22.4	22.7
Hypothalamus	20.8	20.8
Midbrain	24.2	24.4
Medulla	27.1	27.4

[a] Each value is the mean of two determinations, 3 brain areas per determination.

monoamines in the central nervous system (14).

Summary. Norepinephrine (NE) levels were determined in the hypothalamus, midbrain, and medulla oblongata of rats following exposure to cold of 1°C for 1, 15, and 30 days. In comparison to room temperature controls, hypothalamic levels were slightly increased following 15 and 30 days at 1°C, while levels in the medulla and midbrain were slightly increased only after 30 days of cold-exposure. These increases were unrelated to differences in body temperature, body weight and the water content of brain between cold-exposed and room temperature rats. It is suggested that the increases in brain NE derive from a basis similar to the increases in peripheral tissue catecholamines previously observed by others to follow prolonged cold exposure.

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Evidence for *in Vivo* Activity of Postheparin Plasma Lecithinase in Man* (32625)

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Plasma obtained after intravenous administration of heparin contains enzymatic activity catalyzing the hydrolysis of triglyceride (TG) *in vitro*. This activity is also apparent *in vivo* and results in a decrease in total plasma TG, an increase in products of TG hydrolysis, and a shift of the plasma lipoprotein spectrum from very low density to higher density fractions (1). From prior studies it appeared that no change occurred in total plasma cholesterol or phospholipid (1).

Recent studies have shown that postheparin plasma also contains enzymatic activity catalyzing the hydrolysis of the phospholipids phosphatidyl ethanolamine or phosphatidyl choline (lecithin) *in vitro* (2,3). Hydrolysis occurs at the α' (C-1) fatty acid with the production of β (C-2) lyso derivatives. Results in the present study indicate that lipoprotein lecithin is hydrolyzed in parallel with TG *in vivo* after intravenous heparin, and thus provide evidence for the *in vivo* action of postheparin plasma phospholipase in man.

Methods. Blood samples were obtained from 12 normal adult male subjects prior to and again after rapid intravenous administration of 5000 units of heparin. In three additional experiments blood was obtained prior to and 20 and 40 min after 10,000 units of IV heparin. Pre- and postheparin bloods were immediately placed in tubes containing sodium oxalate (1 mg/ml) and sufficient diethyl-*p*-nitrophenyl phosphate (Paraoxon) to effect a concentration of 2×10^{-3} *M* (4). Paraoxon was used to inhibit postheparin lipolytic activities and also the fatty acid acyl transferase described by Glomset (3,4). The use of this inhibitor is critical for docu-

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