Pharmacology Biochemistry & Behavior, Vol. 16, pp. 361–364, 1982. Printed in the U.S.A.

Pyridoxine Reduces Core Body Temperature in Rats

KRISTIN A. LINDSETH AND ROBERT A. HICKS

Department of Psychology, San Jose State University, San Jose, CA 95192

Received 9 June 1981

LINDSETH, K. A. AND R. A. HICKS. *Pyridoxine reduces core body temperature in rats.* PHARMAC. BIOCHEM. BEHAV. **16**(2) 361–364, 1982.—A mild hypothermia was produced in female rats during treatment with pyridoxine HCl (Vitamin B_6), 100 mg/kg, administered in the drinking water. The hypothermic effect appeared by day 3 and persisted through 15 days of treatment. The reduction in core temperature was greater early in the day, just following the nocturnal period of maximum food and water consumption of the rat. Tail tendon temperatures of control and pyridoxine-treated animals showed no evidence of increased heat loss. Thus the hypothermia appears to reflect decreased heat production. The implications of a reduced metabolic rate for gerontological research are discussed.

Pyridoxine Vitamine B_6 Rate of living Hypothermia

RATE of Living theories, which predict a direct relationship between metabolic rate and lifespan are strongly supported by direct evidence from temperature manipulation experiments in poikilotherms. Raising the ambient temperature reduces the lifespan of *Drosophila melanogaster* [11] and trout [16]. Further, exposure of *Drosophila* to higher temperatures results in increased oxygen consumption, acceleration of fine structural aging and shortening of life span.

Drastic temperature manipulation in homeotherms is not easily achieved because of the efficacy of their thermoregulatory mechanisms. However, moderate reductions in core body temperature of homeotherms can be accomplished independent of the environmental temperature by chemical means [6,9]. The studies accomplished to date reveal the need for a chemical suitable for long term use.

Most attempts to induce hypothermia chemically have involved drugs which exert effects on central monoamine systems [6,9]. Evidence for the role of hypothalamic monoamine systems in thermoregulatory processes was obtained in the experiments of Feldberg and Myers [3] who demonstrated that serotonin (5-HT), injected into the cerebral ventricles, evoked an increase in core body temperature and that adrenalin and noradrenalin (NA) produced a decrease, especially when temperature had first been raised by pyrogens or serotonin (5-HT). Both the warming and cooling effects are dose-dependent. Feldberg and Myers proposed that presynaptic release of 5-HT within the anterior hypothalamus activates a heat production pathway, and that NA inhibits heat production by blocking cholinergic synapses [14].

The effect was localized in the anterior hypothalamus by Villablanca and Myers [17]. When pyrogens were injected into the third ventricle, by which the anterior hypothalamus is reached, or directly into the anterior hypothalamus, fever occurred. Injections into other areas of the hypothalamus did not produce fever.

Chlorpromazine, a dopamine receptor blocking agent and the prototype phenothiazine, was described in 1952 by French surgeon Laborit and coworkers as a drug for inducing artificial hibernation. At threshold doses for hypothermia chlorpromazine profoundly inhibits spontaneous motor activity (including eating and drinking) of mice [9]. In addition, it is impossible to induce a mild hypothermia with this drug as the effect is an all-or-none response of a drop of 9–12°C. This may not be a therapeutic reduction as impairment of learning in mice has been observed when body temperature drops more than 2°C [1]. Furthermore tolerance developed within 4 days.

Reserpine, an extract from the roots of *Rauwolfia serpentina*, disrupts presynaptic storage of catecholamines and 5-HT, rendering the monoamines more susceptible to attack by the enzyme monoamine oxidase. Depletion of central monoamines lasts approximately 30 hours. Huge doses are required to produce hypothermia (i.e., 7–14 times the normal therapeutic dose), while even therapeutic doses produce side effects such as depression severe enough to lead to hospitalization, extrapyramidal effects, tendency to sleep, and indifference to environmental stimuli. In addition, reserpine blocks secretion of hypothelamic regulatory hormones.

Paracholorphenylalanine (PCPA) inhibits tryptophan hydroxylation, the rate-limiting step for 5-HT synthesis [7] and may also decrease central NA levels by inhibiting phenylalanine and tyrosine hydroxylase [8]. PCPA treatment produced intermittent hypothermia in mice [6]. Depletion of central 5-HT stores is significant for about 8 days [10] yet hypothermia was intermittent.

The hypothermic agents utilized to date present drawbacks in terms of toxicity at threshold doses, development of tolerance, and extreme side effects. Two chemicals have been found which show no sign of toxicity or side effects at doses producing hypothermia.

Levodopa, injected subcutaneously, reduces temperature

by approximately 1°C. The effect persists longer than that produced by the drugs mentioned above (excepting Reserpine) [6].

Pyridoxine (Vitamin B_6) appears to be the safest potential hypothermic agent. The water-soluble vitamins are relatively non-toxic and Vitamin B_6 can be administered orally. Pyridoxine is converted to pyridoxal phosphate in humans and in rodents. Pyridoxal phosphate is a coenzyme for DOPA decarboxylase which converts L-dopa to dopamine [5]. Intravenous injections of Pyridoxine increase the level of dopamine in the basal ganglia from 6.66 to 8.09 μg/g by 12 hours post injection [15]. Dopamine is converted to norepinephrine which has been shown to exert a cooling effect when injected intraventricularly or into the hypothalamus. Pyridoxine does not appear to have been tested for hypothermic effect. We therefore investigated the effect of a chronic oral dose of 100 mg/kg on the core body temperature of rats.

METHOD

Animals

Twenty-four female Sprague Dawley rats (400–500 gram retired breeders), obtained from Simonsen Laboratories, Gilroy, CA, were used. The animals were housed 3 per cage in an air conditioned room. Temperature was set for 23°C and fluctuated between 23° and 26°C. Lighting conditions were on at 0900 hrs and off at 2100 hrs.

Procedure

Rectal temperatures were taken once a day at 1100 hrs on 4 days during a 10-day pretreatment period with a Sental model 1200 digital temperature indicator. The thermistor probe was inserted 5 cm into the rectum, while the rat was held gently near the tip of the tail.

Water intake was also monitored during the pretreatment period and the data used to calculate the pyridoxine dose required to ensure a dosage of 100 mg/kg/day in the drinking water of each rat. The concentration was 2 g/liter.

Immediately following the 10-day pretreatment period the animals were randomly divided into control and experimental groups. Durg administration, beginning on day 1, was continuous through day 15. Water for control animals and pyridoxine solution for experimental animals was administered ad lib in bottles shielded from light to prevent the breakdown of the vitamin and was changed 3 times weekly. Rectal temperatures were taken on days 3, 4, 5, 10, 13 and 15, beginning always at 1100 hrs and finishing by 1300 hrs. Temperatures were also taken at 2-hr intervals on day 3 to determine the time course of the effect.

Food and water intake and body weights were recorded throughout the treatment period.

A second experiment was performed to determine whether any change in amount of heat loss through extremities occurred during pyridoxine administration. Tail tendon temperatures were taken as a measure of heat loss. It was believed that this measure would be less subject to error than a skin surface temperature measurement as the thermocouple area of the probe, which would be partially exposed to the air if used to measure skin temperature, can be fully inserted into the tendon. Rectal temperatures were taken at 1100 hrs on day 3 pyridoxine administration, immediately following which tail tendon temperatures were taken. A Yale 25 gauge needle probe affixed to thermocouple wire was inserted 1 cm into a subcutaneous tendon 3 cm from the tail tip and read off a Sentel model 1200 digital temperature indicator, following the method of Everitt, Porter and Steele [2].

RESULTS AND DISCUSSION

The mean core temperature and the standard deviation for each of the measurement days for both groups of animals are listed in Table 1.

The data summarized in Table 1 were analysed using a two by seven factor ANOVA with repeated measures in the second factor (days). Both the main effects for treatments and days were significant, i.e., $F(1,28)=23.36$, $p<0.001$, and $F(6,168)=8.63$, $p<0.001$, respectively. The treatments by days interaction was not significant, $F(6,168)=1.48$. The days for which significant between groups differences were computed are indicated in Table 1. The reason for the somewhat smaller difference on day 7 is unclear.

Overall these data show that pyridoxine produced a clear and relatively persistent hypothermic response. Mean core temperature differences reached a maximum of 1.06°C on day 4. The hypothermic response of the pyridoxine-treated group persisted through day 15, but the mean difference on that day was diminished. Thus it is not clear from the results of this preliminary study whether the vitamin would produce a long term hypothermia.

Mean daily water intake in the experimental group was 29.74 during baseline, 28.11 ml/animal during treatment. Thus the animals received an actual mean dose of 58 mg/440 g rat, slightly more than the 100 mg/kg dose which was planned. Food consumption did not differ from baseline in either group. Body weight in the experimental group diminished to 98% of baseline weight by day 15 treatment. This difference was not statistically significant.

To determine the time course of the effect, on day 3, the temperature of each animal was taken at 0900, 1100, 1300 and 1500 hrs. The mean core temperature and the standard deviation for each measurement time for both groups of animals are listed in Table 2. The data summarized in Table 2 were analysed using a two by four factor ANOVA with repeated measures in the second factor (hours). Both the main effects for treatments and hours were significant, i.e., $F(1,28)=6.22$, $p<0.02$, and $F(3,84)=39.29$, $p<0.001$, respectively. The treatments by hours interaction was not significant, $F(3,84)=2.46$.

As is shown in Table 2, the hypothermic response of the pyridoxine-treated group was present over the entire 8-hr recording period. The mean difference between groups reached a maximum at 1100 hrs. The difference diminished by 1500 hrs which probably reflects the nocturnal drinking pattern of the rat. The temperature cycles over the 8-hr period appeared to be similar for both groups.

It was of interest, in relation to the question of metabolic rate, to determine whether the hypothermia was a consequence of reduced heat production which would signify decreased energy metabolism, or of increased heat loss which, although it may occur passively, would not represent energy savings if heat production remains constant. Heat production and conservation require energy, and require it almost continually, in mammals living in an ambient temperature which is almost always lower than the reference temperature of 37°C. There is evidence that the pathway which transmits thermoregulatory information from the preoptic region of the anterior hypothalamus to the posterior hypothalamus is

TABLE 1

EFFECT OF PYRIDOXINE (100 mg/kg) ON MEAN CORE BODY TEMPERATURE OF RATS

Treatment Days	3*	4*	5*	7	10*	13*	15
Control	37.45±0.92	37.40±0.83	36.49±0.67	36.51±0.54	37.25±0.50	36.73±0.56	36.59±0.58
Pyridoxine	36.72±0.80	36.34±1.24	35.73±0.59	36.23±0.50	36.80±0.69	36.35±0.50	36.35±0.51

*Indicates between group difference is significant, $p<0.05$.

TABLE 2

EFFECT OF PYRIDOXINE (100 mg/kg) ON MEAN CORE BODY TEMPERATURE OF RATS AT 2-HR INTERVALS (THESE TEMPERATURES WERE TAKEN ON DAY 3)

Time of Measurement	900†	1100*	1300	1500
Control	36.44±0.68	37.57±1.06	37.53±0.62	36.49±0.53
Pyridoxine	35.65±0.53	36.74±0.80	36.77±1.47	36.26±0.52

*$p<0.05$; †$p<0.01$.

more concerned with heat conservation than with heat loss. Acetylcholine activity increases in caudal pathways in the hypothalamus in most cases in response to a cold stimulus and in far fewer cases in response to warm stimuli [13]. As a measure of heat loss, tail tendon temperatures were taken 3 cm from the tip of the tail. Mean temperatures were 21.79 (±0.38) and 21.80 (±0.16)°C for control and pyridoxine-treated animals respectively. Thus there was no evidence for increased heat loss. A chronic hypothermia, under these circumstances, would be of interest in gerontological research.

A life span experiment is currently underway in our laboratory in which the long term effect of pyridoxine on core temperature and ability to mobilize energy reserves are also being assessed in a larger group of mice.

The single most effective method known of extending life span, caloric restriction, is accompanied by daily decreases in activity and temperature in mice [12]. Interspecies life span comparisons also provide evidence for the relationship between metabolic rate and life span. In one example, the guinea pig, which has a lifetime caloric energy production rate of 4.2×10^2, lives approximately 7 years, while the rat, which has a higher rate of energy production, (5.7×10^2), lives only 4 [4]. Intersex comparisons also support the relationship. The life span potential of women, known to exceed that of men by some 11 years, may be attributable to a metabolic rate which is 8% slower.

A slowed metabolism may result in increased longevity by reducing the rate of accumulation of intracellular damage incurred during cellular work. The rate of accumulation of lipofuscin-ceroid and of fine structural age changes in *Drosophila melanogaster* is temperature-dependent. And the relationship of this pigmented material to age has been demonstrated by B. L. Strehler and others who have shown an almost linear increase with age of lipofuscin in human heart tissue.

REFERENCES

1. Essman, W. B. and F. N. Sudak. Effect of hypothermia on establishment of an avoidance response in mice. *J. comp. physiol. Psychol.* **56:** 366–369, 1963.
2. Everitt, A. V., B. D. Porter and M. Steele. Dietary caging and temperature factors in the aging of collagen fibers in rat tail tendon. *Gerontology* **27:** 37–41, 1981.
3. Feldberg, W. and R. D. Myers. Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol.* **173:** 226–237, 1964.
4. Hershey, D. Energy, basal metabolism and life expectancy. *Gerontologia* **1:** 245, 1963.
5. Hornkiewicz, O. Dopamine (3-hydroxytyramine) and brain function. *Pharmac. Rev.* **18:** 925–964, 1966.
6. Janoff, A. S. and B. Rosenberg. Chemically evoked hypothermia in the mouse: Toward a method for investigating thermodynamic properties of aging and death in mammals. *Mechs Ageing Dev.* **3:** 335–349, 1978.
7. Knapp, S. and A. J. Mandell. Parachlorophenylalanine—its three phase sequence of interactions with the two forms of brain tryptophan hydroxylase. *Life Sci.* **11:** 761–771, 1972.
8. Koe, B. K. and A. Weissman. p-Chlorophenylalanine, a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499, 1966.
9. Liu, R. K. and R. L. Walford. The effect of lowered temperature on life span and immune and non-immune processes. *Gerontologia* **18:** 363–388, 1978.

10. Miller, F. P., R. H. Cox, W. R. Snodgras and R. P. Maikel. Effects of p-chlorophenylalanine, p-chloro-amphetamine and p-chloro-N-methlamphetamine on rat brain NA, 5-HT and 5-hydroxy-indole-3-acetic acid. *Biochem. Pharmac.* **19:** 435–440, 1969.
11. Miquel, J., P. R. Lundgren, K. G. Bensch and H. Atlan. Effects of temperature on life span, vitality, and fine structure of *Drosophila melanogaster*. *Mechs Ageing Dev.* **5:** 347–370, 1976.
12. Morhardt, J. E. and J. W. Hudson. Daily torpor induced in white-footed mice by starvation. *Nature* **212:** 1046–1047, 1966.
13. Myers, R. D. and M. B. Waller. Differential release of acetylcholine from the hypothalamus and mesencephalon of the monkey during thermoregulation. *J. Physiol.* **230:** 273–293, 1973.
14. Myers, R. D. and T. L. Yaksh. Control of body temperature in the unanaesthetized monkey by cholinergic and aminergic systems in the hypothalamus. *J. Physiol.* **202:** 483–500, 1969.
15. Pfeiffer, R. and M. Ebadi. On the mechanism of the nullification of CNS effects of L-Dopa by pyridoxine in Parkinsonian patients. *J. Neurochem.* **19:** 2175–2181, 1972.
16. Verzar, F. *Lectures on Experimental Gerontology*. Springfield, IL: C. C. Thomas, 1963.
17. Villablanca, J. and R. D. Myers. Fever produced by microinjection of typhoid vaccine into the hypothalamus of cats. *Am. J. Physiol.* **208:** 703, 1965.