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Effect of nifedipine on core cooling in rats during tail cold water immersion

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Abstract

Male rats (450 g, $n = 11$ /group) were heated at an ambient temperature of 42°C until a rectal temperature of 42.8°C was attained. Rats, then received either saline (30°C) + tail ice water immersion ($F + I$) or saline (30°C) + tail ice water immersion + Nifedipine, a peripheral vasodilator, ($F + I + N$) to determine cooling rate effectiveness and survivability. The time to reach a rectal temperature of 42.8°C averaged 172 min in both groups resulting in similar heating rates (0.029°C/min). The cooling rates in group $F + I$ and $F + I + N$ were not significantly different from each other. We conclude that since Nifedipine did not improve cooling rates when combined with fluid + tail ice water immersion, its use as a cooling adjunct does not seem warranted.

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Keywords: Heat stress; Heat stroke; Cooling therapies; Nifedipine

1. Introduction

Cold water immersion is the most commonly used technique of cooling hyperthermic persons experiencing the symptoms of heat stroke (Costrini, 1990; Costrini et al., 1979; Kielblock et al., 1986; O'Donnell and Clowes, 1972). However controversy exists whether the peripheral vasoconstriction induced by cold water immersion suppresses heat loss. Evidence for this includes reports that cold water immersion induces similar or even slower rates of core temperature reduction compared to other cooling techniques despite greater core-to-water temperature gradients (Costrini et al., 1979; Holman, 1969; Noakes, 1986). Such data are difficult to interpret, however, because immersion in cold water may induce shivering and physical arousal which act to elevate heat production (Keatinge, 1960; Toner and McArdle, 1996). One approach to eliminate

these confounding factors and examine the role of peripheral vasoconstriction on cooling rates is to employ a non-shivering hyperthermic rat model in which the rat is restrained and the tail (organ of heat exchange, Dawson and Keber, 1979) is immersed in cold water.

The rat is an appropriate model (Moran et al., 1999) to investigate thermal responses because its tail, like human skin (Previte, 1983), markedly vasodilates in response to heat stress (Magazanik et al., 1980; O'Leary et al., 1985) and exhibits vasoconstriction when exposed to cold (Costrini et al., 1979; Hellstrom, 1975; Thomas et al., 1994). Although the direct relationship between rat tail and whole human body cooling cannot be made, causal associations can be made. For instance, because of the high proportion of cutaneous tissue in the tail, thermoregulatory reflex control of blood flow in the tail, analogous to skin blood flow in man, is an important response component to environmental heating and cooling.

The purpose of this study was to determine if peripheral vasoconstriction induced by cold water immersion reduces cooling rates in hyperthermic rats.

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A systemic vasodilator (Nifedipine) was employed to attenuate the vasoconstriction of cold water immersion while maintaining a constant core-to-water gradient. Because of concern that the vasodilator might induce reduced tail perfusion, delay heat loss and make it more difficult to maintain cardiac output due to the additional reduction in peripheral resistance, the additional use of saline infusion to sustain cardiac output was combined with the vasodilator. It was hypothesized that administration of nifedipine would increase body-cooling rates during conditions when tail perfusion was sustained by increasing circulatory volume via saline infusion.

2. Materials and methods

Adult male Sprague-Dawley rats (470 ± 25 g; Charles River Breeding Laboratories, Wilmington, MA) were housed singly in wire bottomed cages for at least 1 week prior to use. Food and water were available ad libitum and the general health of the animals was monitored daily. Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and catheterized (external jugular) using cannulas made of silicone ethylene. Catheterized rats were then allowed 3 days to recover. All rats in the control and experimental groups were similarly catheterized, and studied following an overnight fast with water given ad libitum.

2.1. Procedures

On the experimental day (day 5 post-surgery) animals were placed in a restraining cage and core body temperature was obtained by inserting a thermistor probe (YSI Model 423, Yellow Springs, OH) into the rectum to a depth of 6 cm. Following a blood sample, rats were placed in a heating chamber maintained at $42.0 \pm 0.3^\circ\text{C}$. Rectal temperature (T_{re}) was monitored throughout the heating. Rats were removed when T_{re} reached 42.8°C (Hubbard et al., 1977). Rats were then immediately transferred to a chamber ($30.0 \pm 0.5^\circ\text{C}$), weighed, placed in restraining cages and randomly assigned one of the following treatment groups: (1) fluid administration, vehicle only and ice water immersion (tail) ($n = 11$) ($F + I$) and (2) fluid administration, nifedipine in vehicle and ice water immersion (tail) ($n = 11$) ($F + I + N$). Tail size (15–18 cm length, 1.2 cm diameter) was uniform in all animals.

An intravenous fluid treatment consisting of normal saline (30.0°C) and sodium bicarbonate (0.89 mEq/kg) was administered via the venous catheter. Fluid was replaced and given as a volume equivalent to 80% of the body weight lost during heating. Fluid was infused at a rate of 0.70 ± 0.02 ml/min using a Harvard infusion pump (Harvard Instruments, Cambridge, MA). This procedure was initiated immediately after the heat stress.

Nifedipine (Precardia, Pfizer Laboratories) was selected due to its potent peripheral vascular and least direct cardiac effects and was administered 2 min following the onset of fluid administration at a dose of 0.1 mg/kg. The drug was administered slowly at a rate of approximately 0.05 mg/min into the venous line without interrupting fluid administration. The dose (Falotico et al., 1989) and rate of administration were determined in pilot studies such that the drug caused observable hyperemia in tails and paws without inducing profound systemic hypotension. The vehicle used to dilute the nifedipine was a solution of 40% propylene glycol, 5% ethanol, and 2.5% dextrose. The vehicle was administered to rats in $F + I$ at the same dose and rate as the drug in $F + I + N$.

Tail cold water immersion was then initiated 2 min following the first dose of vehicle or drug. The rat's tail was submerged to within 3 cm of the base in a ice bath maintained at $4\text{--}6^\circ\text{C}$. The tail remained immobile while in the ice water bath. Cold water immersion did not elicit any shivering.

2.2. Data collection

Core temperature was monitored every minute during cooling for 60 min. Rats were then monitored for 24 h survival as well as time to death (for animals surviving less than 4 h).

3. Data analysis

Cooling rates (slope = $^\circ\text{C}/\text{min}$) for each rat were calculated by determining the linear regression between time and temperature from 3 min post-heating to the time of completion of saline administration. Heat transfer due to saline infusion and ambient conditions was calculated according to Van Wylen and Sonntag (1968). Independent *t*-tests were used to determine significance between treatment groups. Statistical significance between cooling rates of each treatment group was determined using an independent *t*-test. A Fishers Exact test was used to detect differences in 24 h survival rates between treatment groups. Significance level was set at $p < 0.05$. Data are presented as mean $\pm$ SE.

4. Results

The time to reach a rectal temperature of 42.8°C averaged 172 min in both groups resulting in similar heating rates ($0.029^\circ\text{C}/\text{min}$). The volume (23.0 ± 7.0 ; 24.6 ± 4.0 ml) and duration (32 ± 5 ; 37 ± 4 min) of fluid administration following hyperthermia was also the same in $F + I$ and $F + I + N$, respectively. The cooling rates in group $F + I$ ($0.074 \pm 0.019^\circ\text{C}/\text{min}$) and $F + I +$

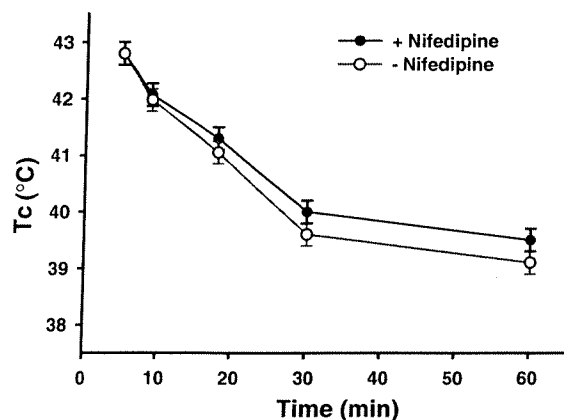


Fig. 1. Change in rectal temperature versus time during recovery from heat stress in response to saline infusion (30°C) and tail ice water emersion (4°C) with and without nifedipine.

N ($0.078 \pm 0.019^\circ\text{C}/\text{min}$) were not significantly different from each other (Fig. 1).

It was calculated that saline administration resulted in a reduction of core temperature of 0.60°C and 0.63°C in $F + I$ and $F + I + N$, respectively. Furthermore, the placement of the rats into a treatment chamber held at 30°C resulted in reducing rectal temperature 1.05°C in both groups. Any further decreases in core temperature (2.20°C and 2.34°C in $F + I$ and $F + I + N$, respectively) are due to tail immersion. Survival rates at 24 h post-treatment $F + I$ (55%; 6/11) and $F + I + N$ (64%; 7/11) were not different ($p < 0.05$).

5. Discussion

This study investigated the effectiveness of the vasodilator nifedipine in conjunction with fluid administration and ice-water immersion of the tail to enhance cooling in a hyperthermic rat model. The rat tail was chosen to study this cooling treatment (Hubbard et al., 1977) since it vasodilates and vasoconstricts in a similar manner as human skin (Shibolet et al., 1976; Thornington, 1966) in response to heat and cold exposure (O'Leary et al., 1985; Raman et al., 1983; Rowell, 1982; Wenger et al., 1975). It was shown that nifedipine, a calcium antagonist, causes similar responses in rat tails (Falotico et al., 1989) as human peripheral tissues since the mechanism of vascular smooth muscle function is calcium dependent for both vascular beds (Schwartz and Triggle, 1984). Our hypothesis was that although the use of cold water immersion along with intravenous fluid was proven to be efficacious in treating heat stroke (Costrini et al., 1979; O'Donnell and Clowes, 1972), nifedipine would blunt cold-induced vasoconstriction

and lead to more rapid core cooling. No previous studies have evaluated whether administration of a systemic vasodilator in conjunction with cold water cooling augments core cooling rates.

The principle finding from this study was that nifedipine administration had no significant effect on core cooling rates. Saline administration combined with removing the rat into a cooler environment accounted for a 1.67°C fall in core temperature while tail cooling via cold water immersion accounted for a 2.27°C fall in core temperature in both nifedipine-treated and untreated rats. These data suggest that the peripheral vasodilatory effect of nifedipine was attenuated by the cold water induced vasoconstriction (Hellstrom, 1975; Rowell, 1982; Thomas et al., 1994) decreasing peripheral blood flow and preventing convective heat transfer from the core to the tail. This lack of response to nifedipine was possibly due to an exaggerated neurally mediated sympathetic outflow (O'Leary et al., 1985; Thomas et al., 1994) as a result of ice water immersion maintaining tail vasoconstriction. This vasoconstrictor response was maintained despite the fact that others (Ferguson and Dorsey, 1985) have shown that nifedipine attenuates vasoconstrictor responses to a cold pressor stimulus and depresses the vasoconstrictor response to intra-arterial infusion of norepinephrine. Alternately it may be argued that nifedipine was able to dilate the peripheral vessels to permit convective heat loss. However this beneficial effect was masked due to the large thermal gradient conducting heat loss through the tail's large surface-to-mass ratio (Thornington, 1966). Finally it is possible that nifedipine was only able to minimally effect vasodilation since Ferguson and Dorsey (1985) demonstrated limited attenuation to a cold pressor test using a higher dose of nifedipine. Thus any convective core cooling in our study would be minimal when compared to conductive heat loss.

Since no significant difference was found in cooling rate when comparing the $F + I$ and $F + I + N$ groups, we conclude that nifedipine provides no additional increase in the rate of convective heat transfer through the tail cutaneous and peripheral vasculature.

Although nifedipine failed to significantly improve cooling rates as well as show significant improvement in survivability, beneficial effects of the calcium antagonist may be gleaned from the fact that vasodilation may be occurring in internal vessels leading to tissues and organs which usually become hypoxic in heatstroke casualties (Wyndham et al., 1959). Furthermore, nifedipine may have a beneficial effect by decreasing muscular tone and heat production (Chinet and Giovannini, 1989) or act by maintaining cellular calcium homeostasis thus promoting cell viability.

In summary, the addition of the systemic vasodilator nifedipine produced no improvement in the cooling rates of hyperthermic rats and its use as an adjunct to induce

more rapid cooling during cold water immersion is not warranted.

Disclaimers

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

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In conducting the research described in this report the investigation adhered to the guidelines outlined in AR 70-18, USARIEM Memorandum 70-18, and the "Guide for the care and use of laboratory animals" (DHEW Publication No. 78-23, Revised 1978).

References

- Chinet, A., Giovannini, P., 1989. Evidence by colorimetry for an activation of sodium hydrogen exchange of young rat skeletal muscle in hypertonic media. *J. Physiol. (London)* 415, 409–422.
- Costrini, A., 1990. Emergency treatment of exertional heat-stroke and comparison of whole body cooling techniques. *Med. Sci. Sports Exer.* 22, 15–18.
- Costrini, A.M., Pitt, H.A., Gustafson, A.B., Uddin, D.E., 1979. Cardiovascular and metabolic manifestations of heat stroke and severe heat exhaustion. *Am. J. Med.* 66, 296–302.
- Dawson, N.J., Keber, A.W., 1979. Physiology of heat loss from an extremity: the tail of the rat. *Clin. Exp. Pharmacol. Physiol.* 6, 69–89.
- Falotico, R., Keiser, J., Haertlein, B., Cheung, W.M., Tobia, A., 1989. Increased vasodilator responsiveness to BRL 34915 in spontaneously hypertensive versus normotensive rats: contrast with nifedipine. *Proc. Soc. Exp. Biol. Med.* 190, 179–185.
- Ferguson, D.W., Dorsey, J.K., 1985. Effects of nifedipine on baroreflex modulation of vascular resistance in man. *Am. Heart J.* 109, 55–62.
- Hellstrom, B., 1975. Cold vasodilation of the rat tail. *Can. J. Physiol. Pharmacol.* 53, 207–210.
- Holman, J.P., 1969. *Thermodynamics*. McGraw-Hill, New York, pp. 73–84.
- Hubbard, R.W., Bowers, W.D., Matthew, W.T., Curtis, F.C., Criss, R.E.L., Sheldon, G.M., Rattree, J.W., 1977. Rat model of acute heatstroke mortality. *J. Appl. Physiol.* 42, 809–816.
- Keatinge, W.R., 1960. The effects of subcutaneous fat and of previous exposure to cold on the body temperature, peripheral blood flow and metabolic rate of men in cold water. *J. Physiol. (London)* 153, 166–178.
- Kielblock, A.J., VanRensberg, J.P., Franz, R.M., 1986. Body cooling as a method for reducing hyperthermia. An evaluation of techniques. *S. Afr. Med. J.* 69, 378–380.
- Magazanik, A., Epstein, Y., Udassin, R., Shapiro, Y., Sohar, E., 1980. Tap water, an efficient method for cooling heat stroke victims—a model in dogs. *Aviat. Space Environ. Med.* 51, 864–867.
- Moran, D., Epstein, Y., Weiner, M., Horowitz, M., 1999. Dantrolene and recovery from heat stroke. *Aviat. Space Environ. Med.* 70, 987–989.
- Noakes, T.D., 1986. Body cooling as a method for reducing hyperthermia. *S. Afr. Med. J.* 70, 373–374.
- O'Donnell Jr., T.E., Clowes Jr., G.H.A., 1972. The circulatory abnormalities of heat stroke. *N. Engl. J. Med.* 287, 734–737.
- O'Leary, D.S., Johnson, J.M., Taylor, W.F., 1985. Mode of neural control mediating rat tail vasodilation during heating. *J. Appl. Physiol.* 59, 1533–1538.
- Previte, J.J., 1983. *Human Physiology*. McGraw-Hill, New York, p. 305.
- Raman, E.R., Roberts, M.F., Vanhuysse, V.J., 1983. Body temperature control of rat tail blood flow. *Am. J. Physiol.* 245, R426–R432.
- Rowell, L.B., 1982. Cardiovascular aspects of human thermoregulation. *Circ. Res.* 52, 367–379.
- Schwartz, A., Triggle, D.J., 1984. Cellular action of calcium channel blocking drugs. *Ann. Rev. Med.* 35, 325–339.
- Shibolet, S., Lancaster, M.C., Danon, Y., 1976. Heat stroke: a review. *Aviat. Space Environ. Med.* 47, 280–301.
- Thomas, J.R., Shurtleff, D., Schrot, J., Ahlers, S.T., 1994. Cold-induced perturbation of cutaneous blood flow in the rat tail: a model of nonfreezing cold injury. *Microvasc. Res.* 47, 166–176.
- Thornington, R.W., 1966. *The Biology of Rodent Tails a Study of form and Function*. Arctic Aeromedical Laboratory, pp. 115–116.
- Toner, M.M., McArdle, W.D., 1996. Human thermoregulatory responses to acute cold stress with special reference to water immersion. In: *Handbook of Physiology, Environmental Physiology*, Vol. 1, M.J. Fregly and C. Blatteis (Eds). American Physiological Society, Sect. 4, Bethesda, MD, pp. 379–397 (Chapter 17).
- Van Wylen, G.J., Sonntag, R.E., 1968. *Fundamentals of Classical Thermodynamics*. Wiley, New York, pp. 82–103.
- Wenger, C.B., Roberts, M.F., Nadel, E.R., Stolwijk, J.A.J., 1975. Thermoregulatory control of finger blood flow. *J. Appl. Physiol.* 38, 1078–1082.
- Wyndham, C.H., Strydom, N.B., Cooke, H.M., Martiz, J.S., Morrison, J.F., Fleming, P.W., Ward, J.S., 1959. Methods of cooling subjects with hyperexia. *J. Appl. Physiol.* 14, 771–776.