ELSEVIER

Neuroscience Letters 339 (2003) 207–210

Neuroscience Letters

www.elsevier.com/locate/neulet

Activation of brown adipose tissue thermogenesis increases slow wave sleep in rat

G. Dewasmes[a,*], N. Loos[a], S. Delanaud[a], D. Dewasmes[a], A. Géloën[b]

[a] *Faculty of Medicine of Amiens, ETPAPC, 3 rue des louvels, 80036 Amiens Cedex, France*
[b] *INSA, U352 INSERM, Domaine Scientifique de la Doua, Bât. L. Pasteur, 11 avenue J. Capelle, 69621 Villeurbanne Cedex, France*

Received 1 November 2002; received in revised form 23 December 2002; accepted 23 December 2002

Abstract

Considering the thermoregulatory role of slow wave sleep (SWS), we wondered whether the sole increase of brown adipose tissue (BAT) thermogenesis could enhance this sleep state. We tested this hypothesis by administering to rats an agonist (BRL 37,344) of the beta-3 adrenoceptor subtype that is massively localized in BAT cell membrane and that is known to activate BAT thermogenesis. Sleep was electrographically characterized. The temperature of interscapular BAT (Tibat) and cortex (Tco) were also assessed. Tibat significantly increased 2–3 h after BRL injection (but not Tco), concomitantly with SWS (+56–57%). At the maximum of Tibat, a significant positive correlation was found between their changes and those of SWS. We demonstrated for the first time that sleep (and especially SWS) can be affected by the specific activation of BAT.

Keywords: Cold defence; Sleep and thermoregulation interaction; Non-shivering thermogenesis; Energy sparing

Brown adipose tissue (BAT) non shivering thermogenesis (NST) is a crucial cold-defence mechanism in the human newborn and in some rodents in adulthood. However, the stimulation of this powerful thermogenic tissue can also strongly deviate the body core temperature (Tc) from euthermia [11,18], including in non-thermal stressing situations [25]. In endothermic Vertebrates, energy expenditure and Tc being lowered during slow wave sleep (SWS), it has been suggested that an increase of this sleep state could be one struggling mechanism against hyperthermia [19]. To our knowledge, the influence that BAT thermal state may exert on sleep is restricted to a study showing that, when it is specifically activated, the heat generated by this tissue can induce an augmentation of active sleep (AS) in newborn rats [26]. However, this interesting finding must be still considered with caution, since a controversy has recently emerged as regards the nature, the estimation (overestimation), and the development of AS in newborns towards its adult form [8]. Considering that SWS could be one mechanism of struggling against hyperthermia, it may thus be asked whether the sole increase of BAT heat production, and/or of its temperature could be correlated with an enhancement of this sleep state. We tested this hypothesis for the first time in rats, notably by taking advantage of the fact that in these rodents the beta-3 adrenoceptor subtypes are massively localized in the cell membrane of brown and white adipocytes [1,9,10].

Seven adult male Wistar rats (IFFA CREDO), were individually caged and housed in a light (light on: 06:00 – light off: 18:00) and temperature (24–25 °C) controlled sound-proofed climatic chamber with free access to a standard diet (Standard chow UAR, AO4) and water. Experiments and installations were approved by governmental authorities (French Ministry of Agriculture, Fishing and Alimentation and Departmental Veterinary Agency of Picardy). All animals were treated according to guidelines approved by the European Communities Council (Directive of 24 November 1986, 86/609/EEC). Each rat was implanted under pentobarbital anaesthesia (50 mg/Kg IP) with EEG and EMG electrodes. A thermistor probe (Shibaura, precision ± 0.10 °C) was inserted under the main locus of BAT (in the interscapular zone) to avoid damaging it. A second probe was placed over the parietal cortices (over the dura) indicating thermal changes occurring in the brain. A very flexible and thin cable

* Corresponding author. Fax: +33-322-827-898.
E-mail address: gerard.dewasmes@sa.u-picardie.fr (G. Dewasmes).

0304-3940/03/$ - see front matter © 2003 Elsevier Science Ireland Ltd. All rights reserved.
doi:10.1016/S0304-3940(03)00018-1

(carefully counterbalanced) was connected between the head and a rotating swivel (plastic one) to prevent the animal from twisting its cable. Temperatures of the interscapular BAT (Tibat) and of the cortex (Tco) were recorded every 5 s on an 80-channel analogic-digital station (AOIP). Sleep was recorded on an Astromed/Grass analogic-digital polygraphic station (Model 15) and scored in periods of 30 s as wakefulness (W), SWS or paradoxical sleep (PS). A recovery period from surgery of 2 weeks was observed before the experiment began. We studied the hypnic and thermal responses to an injection (i.p.) of a highly specific (BRL 37,344) beta-3 adrenoceptor agonist [7]. Since the rat sleeps most of the time during the diurnal phase, we studied the effects of this agonist during the dark period to avoid a ceiling effect (injection just before light off). Knowing that changes of central temperatures also influence sleep [24], we researched from preliminary tests, and chose a single dose increasing BAT temperature, and not that of brain, i.e. 1 mg/kg (dissolved in saline). This dose was clearly shown to stimulate BAT NST in rats [2]. Each rat received an injection of either saline (reference, S) and of the agonist (BRL) at a one week interval. Three rats were first injected with S and then with BRL, this order was reversed in the four others. Recordings began at light off. Just before the first injection (either of S or BRL) two 24 h recordings were performed to ensure the stability of thermal and hypnic variables presently studied under S, and the effect of injection per se. Two-way analysis of variance (agonist × time) for repeated measurements, and Student's *t*-tests, were used to test for statistical significance of the results. We correlated changes in temperature levels and those of sleep by using linear regressions. All means are given ± SEM.

The effect of BRL injection on hypnic and thermal variables was transitory and clearly restricted to the first half of the night. The hourly mean levels of Tibat were significantly ($F1/6$: 18.22; $P < 0.01$) changed by BRL injection during this period, but not those of Tco ($F1/6$: 3.37; ns) (Fig. 1C,D). Tibat was significantly increased between 19:00 and 21:00, the maximum of deviation (+0.5 °C vs. S) being reached 3 h after the injection of the agonist (Fig. 1C). At this time, the individual values of the hourly mean levels of Tco and Tibat observed in S and BRL conditions were correlated ($r = 0.71$, $P < 0.01$).This agonist also provoked significant ($F1/6$: 11.18; $P < 0.05$) modifications of the hourly mean level of SWS during the first 6 h of the night, essentially at the expense of W ($F1/6$: 10.31; $P < 0.05$) (Fig. 1A,B). SWS was significantly augmented by 57% between 19:00 and 20:00, and by 56% during the following hour (Fig. 1A). PS was not statistically changed by the BRL injection. The individual values of the hourly mean levels of thermic and hypnic variables

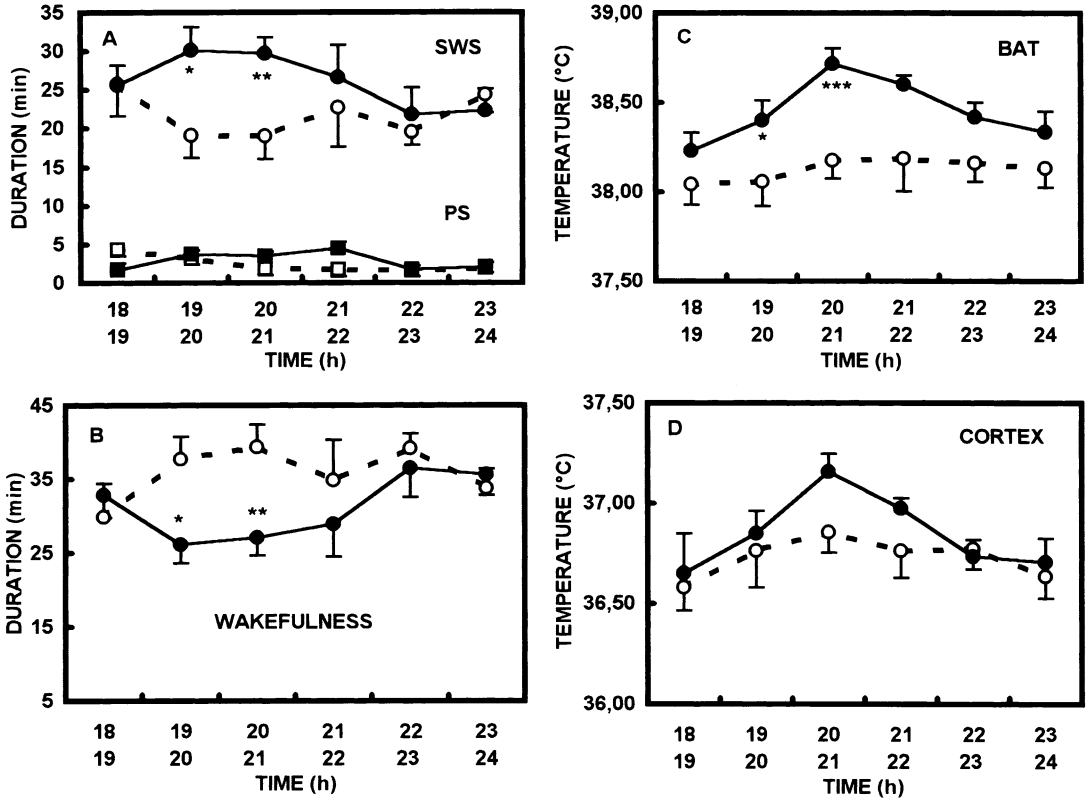

Fig. 1. Effects of S (open circles or open squares and dashed lines) and BRL (full circles or full squares and full lines) injections (just before light off: 18:00) on the hourly mean durations of SWS (A), PS (A) and wakefulness (B) and on the hourly mean levels of BAT (C) and cortex (D) temperatures during the first 6 h of the night. SEM are indicated by vertical bars. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Student's paired *t*-tests: S vs. BRL).

observed in S and BRL conditions were also correlated. Although Tibat and SWS changes significantly increased between 19:00 and 20:00 we found no correlation. However, between 20:00 and 21:00, the relationship between these two variables appeared consolidated since they were linearly ($y = 11,32\ x - 411,63$) and significantly correlated ($r = 0.54$, $P < 0.05$). No correlation was found for PS.

The activation of BAT beta 3 adrenoceptors by a specific agonist provoked, as expected, a significant transient increase of Tibat and, as hypothesized, a concomitant augmentation of SWS. These two events being correlated, we point up for the first time that BAT activation could influence the hypnic function of rats sleeping under standard conditions of temperature and diet, thus excluding the influence of these environmental factors also known to respectively affect SWS [23] and NST [12].

A clear increase of Tibat was observed after BRL injection. Since it has been well established that the stimulation of beta-3 adrenoceptors by a specific agonist increases the NST produced by BAT cells [2,13,31] it is conceivable that the heat produced underlay this Tibat increase. However, possible modulating influences of the vasomotor function must be also considered, notably because the stimulation of beta-3 adrenoceptors could vasodilate vessels of peripheral heat exchangers [5,15]. It may be indeed thought that such a vasodilation would limit Tibat increase, notably by supplying BAT with cooler blood coming from these exchangers. However, by keeping the animals within the thermoneutral zone, this cooling effect was probably marginal. Moreover, to date, the influence that these heat exchangers may exert on BAT thermal state during sleep was clearly demonstrated only during PS spent at low Ta [6,17]. In other respects, it has been reported that BAT vessel vasodilation is partly under the control of beta-3 adrenoceptors [28]. However, this vasomotor effect was not as sensitive as the thermogenic one, since a vasodilation, that limits a Tibat increase by augmenting the thermal clearance through the tissue, only occurred when the agonist was injected at high pharmacological doses [28]. To sum up, even though influences exerted by the vasomotor tone could not be completely ruled out, the consequences for BAT thermal state were likely quite marginal in our experimental conditions.

The possibility that the agonist acts on beta-3 adrenoceptors located in the brain remains open to discussion since, originally not identified in the brain, recent studies using reverse transcription-polymerase chain reaction have detected the presence of beta-3 adrenoceptor mRNA in cerebellum, striatum and hippocampus [27]. This latter central structure was notably involved in the control of BAT heat production, but not through beta-3 adrenergic mechanisms [16]. Thus, the present hypnogenic response was probably not mediated by beta-3 adrenoceptors located in the brain.At the central level, it has been well shown that the hypothalamic thermostat controlled BAT thermogenesis [29,30]. Moreover, the thermal status of the hypothalamic thermostat is also known to modulate SWS changes, either directly [20,24] and/or in relation with the tonic vasoconstrictor sympathetic outflow to heat exchangers [3]. Thus, it may be thought that thermal changes occurring in this integrative structure could be at the origin of the correlation observed between SWS and Tibat changes. Knowing that cortical and hypothalamic temperatures can vary in parallel [14,21,22], the slight Tco increase observed between 20:00 and 21:00 (correlated with the maximum of Tibat changes) would support this idea. However, our peripheral BRL injection did not provoke any significant change of Tco. During the second hour, the Tco (uncorrelated with those of Tibat) measured in S and BRL conditions were quite similar whereas SWS was already high in BRL condition. Lastly, Tco changes were never correlated with those of SWS, even during the third hour. Thus, it may be thought that under our experimental conditions thermal state changes occurring within the hypothalamic thermostat were not primarily involved in the present hypnogenic response. However, this remains to be demonstrated by directly measuring hypothalamic temperature variations because they can diverge from those of cortex due to differences in local heat losses.

In conclusion, a specific BAT activation can affect sleep, and especially SWS. The promotion of SWS probably limits energy wasting from BAT and/or possibly refrains the development of hyperthermia. This hypnic response is also congruent with the hypothesis stipulating that one function of SWS could be to spare energy [4].

Acknowledgements

This work was supported by the Regional Council of Picardy, France.

References

[1] J.R. Arch, A.J. Kaumann, Beta-3 and typical beta3-adrenoceptors, Med. Res. Rev. 13 (1993) 663–729.
[2] C. Atgié, G. Faintrenie, C. Carpéné, L.J. Bukowieki, A. Géloën, Effects of chronic treatment with noradrenaline or a specific beta-3-adrenergic agonist, CL 316243, on energy expenditure and epididymal adipocyte lipolitic activity in rat, Comp. Biochem. Physiol., A 119A (1998) 629–636.
[3] A. Azzaroni, P.L. Parmeggiani, Synchronized sleep duration is related to tonic vasoconstriction of thermoregulatory heat exchangers, J. Sleep Res. 4 (1995) 41–47.
[4] R.J. Berger, N.H. Phillips, Energy conservation and sleep, Behav. Brain Res. 69 (1995) 65–73.
[5] M. Berlan, J. Galitzky, A. Bousquet-Melou, M. Lafontan, J.L. Montastruc, Beta-3 adrenoceptor-mediated increase in cutaneous blood flow in the dog, J. Pharmacol. Exp. Ther. 268 (1994) 1444–1451.
[6] M. Calasso, E. Zantedeschi, P.L. Parmeggiani, Cold-defense function of brown adipose tissue during sleep, Am. J. Physiol., (Regulatory Integrative Comp. Physiol.) 265 (1993) R1060–R1064.
[7] L.J. Emorine, F. Bruno, J. Pairault, M. Briend-Sutren, C. Nahmias, S.

Marullo, C. Delavier-Klutchko, D.A. Strosberg, The human B3-adrenergic receptor: relationship with atypical receptor, Am. J. Clin. Nutr. 55 (1992) 215S–218S.
[8] M.G. Frank, H.C. Heller, Development of REM and slow wave sleep in the rat, Am. J. Physiol. (Regulatory Integrative Comp.Physiol.) 272 (1997) R1792–R1799.
[9] A. Géloën, P. Trayhurn, Regulation of the level of uncoupling protein in brown adipose tissue by insulin, Am. J. Physiol., (Regulatory Integrative Comp. Physiol.) 258 (1990) R418–R425.
[10] J.G. Granneman, Noradrenaline and BRL 37344 stimulate adenylate cyclase by different receptors in rat brown adipose tissue, J. Pharmacol. Exp. Ther. 254 (1990) 508–513.
[11] J. Himms-Hagen, Nonshivering thermogenesis, Brain Res. Bull. 12 (1984) 151–160.
[12] J. Himms-Hagen, Role of brown adipose tissue thermogenesis in control of thermoregulatory feeding in rats: a new hypothesis that links thermostatic and glucostatic hypothesis for control of food intake, Proc. Soc. Exp. Biol. Med. 208 (1995) 159–169.
[13] J. Himms-Hagen, J. Cui, E.J. Danforth, D.J. Taatjies, S.S. Lang, B.L. Waters, T.H. Claus, Effect of CL 316,243, a thermogenic B-3 agonist, on energy balance and brown and white adipose tissues in rats, Am. J. Physiol. (Regulatory Integrative Comp. Physiol.) 266 (1994) R1371–R1382.
[14] H. Kawamura, D.I. Whitmoyer, C.H. Sawyer, Temperature changes in the rabbit brain during paradoxical sleep, Electroenceph. Clin. Neurophysiol. 21 (1966) 469–477.
[15] J.S. Lacroix, A.M. Kurt, S. Auberson, C. Bretton, Beta-3 adrenergic mechanisms in the nasal mucosa vascular bed, Eur. Arch. Otorhinolaryngol. 252 (1995) 298–303.
[16] M. Monda, A. Papa, G. Brizzi, B. DeLuca, Neostigmine in the hippocampus increases thermogenesis and T3-to-T4 conversion, Am. J. Physiol. (Regulatory Integrative Comp. Physiol.) 270 (1996) R1215–R1219.
[17] J. Narebsky, E. Tegowska, New Aspects of temperature relugation during sleep, in: W.P. Koella, F. Obal, H. Schulz, P. Visser (Eds.), Sleep “86”, Fisher Verlag, Stuttgart, 1988, pp. 62–64.
[18] D.G. Nicholls, R.M. Locke, Thermogenic mechanisms in brown fat, Physiol. Rev. 64 (1984) 1–64.
[19] F. Obal Jr, Thermoregulation and sleep, Exp. Brain Res. 8 (1984) 157–172.
[20] F. Obal Jr, P. Alföldi, G. Benedek, F. Obal, Possible contribution of sleep to heat defense, in: W.P. Koella, F. Obal, H. Schulz, P. Visser (Eds.), Sleep “86”, 86, Springer Verlag, Stuttgart, 1988, pp. 58–61.
[21] F. Obal Jr, G. Rubicsek, P. Alfoldi, G. Sary, F. Obal, Changes in brain and core temperatures in relation to the various arousal states in rats in the light and dark period of the day, Pflügers Arch. 404 (1985) 73–79.
[22] A.J. Rampone, M.E. Shirasu, Temperature changes in the rat in responses to feeding, Science 144 (1964) 317–319.
[23] B. Roussel, P. Turillot, K. Kitahama, Effect of ambient temperature on the sleep-waking cycle in two strains of mice, Brain Res. 294 (1984) 67–73.
[24] S. Sakaguchi, S.F. Glotzbach, H.C. Heller, Influence of hypothalamic temperature and ambient temperatures on sleep in kangaroo rats, Am. J. Physiol. (Regulatory Integrative Comp. Physiol.) 237 (1979) R80–R86.
[25] H. Shibata, T. Nagasaka, Contribution of non shivering thermogenesis to stress-induced hyperthermia in rats, Japanese. J. Physiol. 32 (1982) 991–995.
[26] G. Sokoloff, M.S. Blumberg, Active sleep in cold-exposed infant Norway rats and Syrian golden hamsters: the role of brown adipose tissue thermogenesis, Behav. Neurosci. 112 (1998) 695–706.
[27] R.J. Summers, M. Papaioannou, S. Harris, B.A. Evans, Expression of B3- adrenoceptors mRNA in rat brain, Br. J. Pharmacol. 116 (1995) 2547–2548.
[28] H. Takahashi, S. Nakamura, H. Shirahase, T. Yoshida, M. Nishimura, M. Yoshimura, Heterogenous activfity of BRL 35135, a beta-3 adrenoceptor agonist, thermogenesis and incresased blood flow in brown adipose tissue in anaesthetized rats, Clin. Exp. Pharmacol. Physiol. 21 (1994) 539–543.
[29] J. Thornhill, I. Halvorson, Brown adipose tissue thermogenic responses of rats induced by central stimulation: effects of age and cold acclimation, J. Physiol. (London) 426 (1990) 317–333.
[30] J. Thornhill, A. Jugnauth, I. Halvorson, Brown adipose tissue thermogenesis evoked by medial preoptic stimulation is mediated via the ventromedial hypothalamic nucleus, Can. J. Physiol. Pharmacol. 72 (1994) 1042–1048.
[31] J. Zhao, B. Cannon, J. Nedergaard, Thermogenesis is beta3- but not beta1-adrenergically mediated in rat brown fat cells, even after cold acclimation, Am. J. Physiol. (Regulatory Integrative Comp. Physiol.) 275 (1998) R2002–R2011.