Biological Sciences

Pak J Sci Ind Res 1999 42 (1) 27-29

Caffeine Injection in the Dark Phase Prolongs the Nocturnal Rise in Serotonin *N*-Acetyltransferase Activity and Melatonin Content in the Pineal Gland of Male Rats

Ismail Sabry

Zoology Department, Faculty of Science, Kuwait University, P.O. Box 5969, Safat-13060, Kuwait

(Received 1 December 1994; accepted 18 August 1997)

Caffeine, an important member of methylxanthines, induced a prolonged nocturnal rise in pineal melatonin content and an increase in its rate-limiting enzyme serotonin *N*-acetyltransferase (NAT) activity. The highest levels were reached five hours after subcutaneous caffeine injection to male rats in the dark phase, where the NAT activity increased from 920±70 pM pineal^{-1}h^{-1} in the control group to 1190±120 pM pineal^{-1}h^{-1} ($p<0.001$) in the treated group. The pineal melatonin content, as well, was elevated from 520±40 pg pineal^{-1} in the control group to 1120±80 pg pineal^{-1} ($p<0.001$) in caffeine treated group. These changes could be attributed to the depressive effect of caffeine on the activity of phosphodiesterase (PDE), the enzyme responsible for the hydrolysis of the intracellular second messenger cyclic adenosine monophosphate (cAMP).

Key words: Caffeine, Pineal gland, Melatonin.

Introduction

Photic information is transferred from retinas of the lateral eyes to the pineal gland. The nerve endings which terminate within the vicinity of the pinealocytes secrete, primarily during darkness, a neurotransmitter which acts on the cells to stimulate their synthetic machinary (Reiter 1983). The neurotransmitter is generally considered to be norepinephrine based on the studies of Zatz (1981) using rats. Norepinephrine acts on β-adrenergic receptors on the pinealocyte membrane; this stimulates a series of events in which cyclic 3', 5'-adenosine monophosphate (cAMP) serves as a second messenger in the production of a number of pineal hormones (Oleshansky and Neff 1978). The major hormonal product of the pineal gland seems to be melatonin (Reiter and Vaughan 1977).

Phosphodiesterase (PDE) represents the only known catabolic pathway for cyclic nucleotides (Butcher and Sutherland 1962; Strada *et al* 1984). There are various forms of cyclic nucleotide PDE (Wells and Hardman 1977), type IV PDE is the cAMP-specific enzyme (Strada *et al* 1984; Wells and Hardman 1977; Chasin and Harris 1976). Methylxanthines have a large number of biological effects, mainly ascribed to an inhibition of cAMP breakdown (Bergstrand 1980; Silva *et al* 1986). Caffeine, a central nervous system stimulant (Rall 1980) is one of the most important members of methylxanthines because of the widespread use of caffeine-containing beverages such as tea and coffee.

In the present study, the effect of caffeine on the nocturnal rise of pineal NAT activity and the increase of pineal melatonin content in male rats is studied.

Materials and Methods

Sixty male Sprague-Dawley albino rats that averaged 150g in body weight (Purchased from MRI, Alexandria, Egypt) were used in the present study. The animals were housed, 5 per cage and supplied with food and drinking water *ad libitum,* in an environmentally controlled room (temperature was adjusted at 23±2°C) illuminated (flurescent Super Saver, 300-500 lux) daily from 06:00h on a light:dark schedule of 14:10 for 14 days prior to the experiment. All injections and decapitations of animals were carried out under safe dim red light.

On the night of the experiment, animals were divided into two groups (30 animals each). The first group was subcutaneously (sc) injected with 2mg caffeine animal^{-1} in 100 µl saline. Caffeine was purchased from Sigma (St. Louis, M0, USA). The second group served as control. All injections were carried out in the dark phase between 22:45-23:00h.

At three time points, 24:00, 02:00 and 04:00h, 10 animals from each group were rapidly decapitated. Pineal glands were dissected out, individually frozen and homogenized in 100µl of 0.05M phosphate-buffered saline (pH 6.8). Glands were individually assayed for NAT activity using the procedure outlined by Champney *et al* (1984) and melatonin content was

estimated using a radioimmunoassay outlined by Rollag and Niswender (1980). Data represented as mean ± SE were statistically analyzed using a Student's t-test.

Results and Discussion

The pineal gland, an end organ of the visual system, translates the photoperiodic message into a chemical signal (melatonin) which serve as a messenger to every organ in the body (Reiter 1991a). In rats, normally, a nocturnal melatonin peak occures near the mid-dark phase and the levels of melatonin reaches the daytime levels by the end of the dark phase (Reiter 1991b). In the present study, caffeine-treated male rats showed a higher NAT activity than their corresponding saline injected controls at all of the studied time points (Fig 1). However, the difference in activity was found to be statistically significant only at 04:00h, i.e. 5 h after injection (Fig 1). While the NAT activity in the control group was 920±70 pM pineal^{-1}h^{-1} (Fig 1), the NAT activity in caffeine treated animals was still increasing and reached a value of 1190±120 pM pineal^{-1}h^{-1} ($p<0.001$) (Fig 1). Almost a similar pattern for the pineal melatonin content was observed after caffeine injection to male rats (Fig 1). A significant increase from 520±40 pg pineal^{-1} in the control group (Fig 1) to 1120±80 pg Pineal^{-1} in the control group (Fig 1) to 1120±80 pg pineal^{-1} ($p<0.001$) in the treated group was recorded at 04:00h (Fig 1).

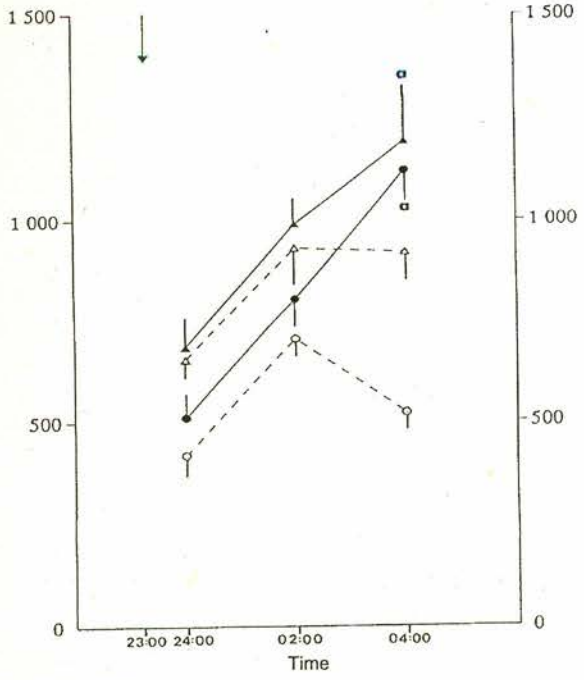

Fig 1. Pineal NAT activity and melatonin content after caffeine treatment (arrow: time of injection), a: $p<0.001$ vs corresponding control.

In the pineal gland norepinephrine is secreted from the nerve endings during darkness (Zatz 1981); where it activates adenylate cyclase at the pinealocyte membrane to form cAMP (Oleshansky and Neff 1978). It is well known that cAMP concentration is rapidly increased after hormonal stimulation, but that increase is short lived *in Vivo* (Deguchi 1973). In the pineal gland, accumulation of cAMP is associated with the induction of NAT (Deguchi and Axelrod 1972).

Caffeine, theophylline and other methylxanthines have a large number of effects commonly ascribed to an inhibition of cAMP breakdown (Bergstrand 1980; Silva 1986). In organ-cultured pineal glands, theophylline was found to raise the concentration of cAMP (Strada *et al* 1972).

Despite the original finding by Butcher and Sutherland (1962) and the results reported by others (Bergstand 1980; Silva *et al* 1986; Strada *et al* 1972) supporting that methylxanthines are inhibitors of PDE - the enzyme which catalyzes the hydrolysis of cAMP to 5'AMP-there is some argument about that assumption (Fredholm 1980; Green and Stiles 1986). However, the data reported in the present work shows that caffeine induces a sustained nocturnal peak in both pineal NAT activity and melatonin content.

Acknowledgment

The author is greatly indebted to Prof. R.J. Reiter, the University of Texas, Health Science Center at San Antonio, Texas, USA for his kind support. The technical assistance of the members of the UNARC, Egypt is greatly appreciated.

References

Bergstrand H 1980 *Eur J Respir Dis* (suppl 109) **61** 37.

Butcher R W, Sutherland E W 1962 *J Biol Chem* **237** 1244.

Champney T H, Holtorf A P, Steger M, Reiter R J 1980 *J Neurosci Res* **11** 59. Chasin M, Harris D N 1976 *Adv Cyclic Nucleotide Res* **7** 225.

Deguchi T, Axelrod 1972 *J Proc Natl Acad Sci USA* **69** 2547.

Deguchi T 1973 *Mol Pharmacol* **9** 184.

Fredholm B B 1980 *Trends Pharmacol Sci* **1** 129.

Green R M, Stiles G J 1986 *Clin Invest* **77** 222.

Oleshansky M A, Neff N H J 1978 *Neural Transm Suppl* **13** 81.

Rall T W 1980 In: *The Pharmacological Basis of Therapeutics*, eds Goodman G A, Goodman L S & Gilman A. New York: Macmillan pp 592-607.

Reiter R J Vaughan M K 1977 *Life Sci* **21** 159.

Reiter R J 1983 In: *Plant, Animal and Microbial Adaptations to Terrestrial Environment*, eds Margaris N S, Arianoutsou-Fraggitaki M & Reiter R J. Plenum Publishing Corp pp 193-201.

Reiter R J 1991a *Moll Cell Endocrinol* **79** C 153.
Reiter R J 1991b *Trends Endocrinol Metab* **2** 13.
Rollag M D, Niswender G D 1980 *Endocrinology* **98** 482.
Silva W I, Shook W, Mittag T W, Ruszkin S 1986 *J Neurochem* **46** 1236.
Strada S J, Klein D C, Weller J, Ewiss B 1972 *Endocrinology* **90** 1470.
Strada S J, Martin M W, Thompson W J 1984 *Adv Cyclic Nucl Prot Phosphor Res* **16** 13.
Wells J N, Hardman J G 1977 *Adv Cyclic Nucleotide Res,* **8** 119.
Zatz M 1981 In: *The Pineal Gland Vol. I. Anatomy and Biochemistry*, ed Reiter R J. CRC Press, Boca Raton pp 229-242.