The Effect of Melatonin Treatment and Exposure to Continuous Darkness on the Reactivity of Smooth Muscles to Drugs in Rats

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الخلاصة

الظلام المطوَل له تأثيرات فسلجية وكيميائية ومرضية على أعضاء وأجهزة ووظائف الجسم المتنوعة. أجريت هذه الدراسة على الحيوانات المختبرية (الجرذان) بقصد التحري عن التأثيرات المحتملة للتغيرات البيئية على تفاعليات العضلة الملساء للقنوات الدافقة في المجاميع المتشابهة الأعمار بالإضافة إلى استعمال مركب الميلاتونين ودراسة تأثيره عليها. تم تقسيم الجرذان إلى ثلاث مجاميع: المجموعة الأولى تم تعريضها إلى ١٢ ساعة من الظلام و١٢ ساعة من الضوء لمدة أربعة أسابيع. المجموعة الثانية حفظت في بيئة مظلمة ولمدة ٤ أسابيع ايضاً. المجموعة الثالثة تم تعريضها إلى ظلام مستمر ولمدة أسبوعين مع حقنها بمركب الميلاتونين خلال الأسبوع الثاني فقط.

وجد ان القنوات الدافقة المأخوذة من المجموعة الثالثة أحدثت زيادة واضحة في تفاعلاتها لعقار النورأدرينالين و5-هيدروكسيتربتامين بالمقارنة مع الأنسجة المأخوذة من حيوانات السيطرة والحيوانات المعرضة للظلام المستمر لمدة ٤ أسابيع, قد تعزى هذه الزيادة إلى التأثيرات المباشرة او غير المباشرة لمركب الميلاتونين من خلال إفراز النورأدرينالين من النهايات العصبية السمبثاوية او قد تحدث بسبب تركيب عدد جديد من المستقبلات.

Abstract

Melatonin is the major secretory product of the pineal gland, playing an integral role in numerous metabolic, physiological and behavioral processes.

This study represents an attempt to understand the effect of environmental changes on the responses of a selected isolated tissue to pharmacologically active substances in certain species which may lead to important experimental and clinical implications in future. Twenty male albino wistar rats were divided into three experimental groups:

Group 1: Control animals, comprising 6 rats were exposed to 12:12 hour light: dark cycle for 4 weeks.

Group 2: Composed of 8 rats, was kept in a dark environment for 4 weeks.

Group 3: Consisted of 6 rats aged 6-7 weeks, subjected to continuous darkness for 2 weeks, at the end of the first week the rats treated daily with s.c melatonin

injection at 1.00 p.m. for 7 consecutive days. The result of this study showed that the vas deferens preparation from the third group reacted with significant increase in reactivity to nor-adrenaline and 5-HT than that of control and continuous dark animals.

The limitation of time and shortage of materials may be blamed for the kind and quantity of results obtained and the conclusions made. Therefore, further studies with regard to the exposure period have to be undertaken in future to extensively elaborate on this point.

Key words: Melatonin, Vas deferens, 5-HT, Nor-adrenaline.

Introduction

Melatonin (N-acetyl 5-methoxytryptamine) is the major secretory product of the pineal gland; it is an indole amine with a 232 molecular weight[1].

Melatonin synthesis and secretion by the pineal gland depends on environmental lighting conditions. It is stimulated by darkness and is inhibited by light. Light prevents the adrenergic activation of the pineal gland resulting in an inhibition of melatonin synthesis[2].

The initial precursor for melatonin is the indole amino acid tryptophan, which is taken up from the plasma by the pineal gland. Tryptophan is hydroxylated in the pinealocyte to 5-hydroxytryptophan by tryptophan hydroxylase. 5-hydroxytryptophan is decarboxylated by aromatic-L-amino acid decarboxylase to become 5- hydroxytryptamine (5-HT) [1, 2]. Within pineal gland, 5-HT is produced only in pinealocytes and some of it is taken up into the adjoining nerve terminals. The enzyme N-acetyltransferase (NAT) converts 5-HT into N-acetylserotonin, which serves as a precursor of melatonin, it becomes melatonin via the action of hydroxyindole-O-methyltransferase (HIOMT)[3].

Nor-adrenaline acts on beta-adrenergic receptors in the membrane of pinealocytes, stimulation of these receptors by the postgangilionic fibers causes an activation of adenylcyclase, leading to increased cAMP. This in turn increases NAT level, causing a rise in melatonin synthesis[4].

Light is considered as the main agent regulating the duration and phase of melatonin synthesis. There is a rapid decline in cAMP and NAT (the key enzyme in melatonin synthesis) following exposure to light or treatment with (propranolol), consequently this leads to reduce melatonin synthesis and secretion by the pineal gland[5].

5-HT is biologically active amine that is found in many tissues and has complex physiologic and pathologic effects. The rat vas deferens contains 5-HT and it has been observe that the tissue concentrations of the amine are higher in regions nearer the prostatic gland than the epididymis. 5-HT has both direct and indirect actions on the rat isolated vas deferens. Direct effects are mediated by an interaction with both tryptamineragic D-receptors and α_1-adrenoceptors,

indirect effect is prevailingly due to the release of nor-adrenaline from sympathetic nerve terminals. Nor-adrenaline has a potent stimulant action on smooth muscle contractility of the rat vas deferens, the contractile effect of nor-adrenaline on the rat isolated vas deferens is more potent than that induced by 5-HT[6].

Materials and Methods

Serotonin creatinine sulphate from B.D.H. and nor-adrenaline bitartrate from winthrop laboratories. A stock solution was prepared in a concentration of (10^{-3} molar solution) and stored in a deep freeze, when required; a dilution was made in kreb's solution. Melatonin: N–acetyl-5 methoxytryptamine., purchased from Sigma chemical co. Solution was obtained by dissolving melatonin in a minimal volume of ethanol and diluting with sterile phosphate saline (PBS) to a final 0.2% ethanol – PBS dilution[3].

Kreb's solution of the following composition was prepared daily and used as the physiological solution in all experiments performed in this study. Quantities required for the preparation of 10 liters: Nacl 69 gm, Kcl 35ml, 10%$MgSo_4 7h_2O$ 29 ml, 10% KH_2PO_4 16 ml, Glucose 20 gm, $NaHCO_3$ 21 gm, 10% $Cacl_2$ 25.2 ml.

The PH was maintained at PH 7.4 by continuously gassing the solution with 95% O_2 and 5% CO_2.

Twenty male albino wistar rats, aged 4-7 weeks, weighing (76-131gm) were maintained in wire – mesh cages, under controlled conditions of temperature ($24 \pm 2C^o$), and were fed with a regular diet and water. The rats were divided into 3 experimental groups[5]:

Group 1: Control animals, comprising 6 rats (weighing 76-92 gm), was exposed to the ordinary photoperiod (day light: darkness cycle 12:12 h) for 4 weeks.

Group 2: Continuous dark animals, composed of 8 rats (weighing 76-92 gm), was kept in a large closed box with few tiny ventilation holes forming a completely dark environment for a period of 4 weeks.

Group 3: Consisted of 6 rats (weighing 110-131 gm), were kept in complete darkness under the same controlled conditions for group 2, for a period of 2 weeks.

At the end of the first week the rats treated daily for 7 consecutive days with s.c injection of 20 *Mg/kg* body weight melatonin at 1.00 p.m. Melatonin solution was freshly prepared as 50 *Mg/ml* [3]. At the end of the predetermined period, the animals were anaesthetized with ether and killed by immediate exsanguinations. The abdomen was rapidly opened, each vas deferens was carefully cleared from all connective tissue surroundings *in vivo* before being cut between the prostatic and epididymal attachment; and then placed in a Petri dish filled with kreb's solution saturated with 95% O_2 and 5% CO_2 mixture. The

vas deferens was then cleared from further connective tissue under the dissecting microscope and was mounted vertically in a 50 ml organ bath containing kreb's solution gassed with the gas mixture and warmed continuously at 37c^{o}.The tissue was left in the organ bath for 30 minutes to equilibrate with 0.5 gm tension on it before nor-adrenaline or 5-HT dosing started[6].

The mechanical activity was recorded isometrically by means of force-displacement transducer, type D-1, 50 gram (George Washington LTD). The instrument was calibrated to give a deflection of 10 mm every 0.5 gm tension.

Doses of nor-adrenaline and 5-HT were added to the organ bath containing the vas deferens preparation and left in contact with the tissue for one minute, and then the tissue was washed three times at 30 seconds intervals [6].

Applied doses were increased geometrically by two-fold each time until a maximum response was achieved. The dose started at (0.5×10^{-5} molar solution) and went up to (4.8×10^{-3} molar solution).

Changes in mechanical activity were plotted against dose to obtain a dose-response curve to 5-HT and nor-adrenaline.

Statistical Analysis

Student's t-test (unpaired-comparison) was used to evaluate the significance of differences among groups. The level of significance was taken as 0.05 or less ($p < 0.05$) [7].

Results

Responses to 5-HT of vas deferens from control (group 1), continuous dark 4 weeks (group 2) and continuous dark 2 weeks + melatonin injection (group 3): In 5-HT dose-response curve, responses to 5-HT were of greater maximal responses in the vas deferens from group 3 compared with those in from group 1. The elevated maximal responses were associated with no such difference at the lower part of the concentration-response curve. The maximal responses ($\pm$ S.E) were (0.358 ± 0.049) for group 3, (0.181 ± 0.021) for group 1 and (0.095 ± 0.026) for group 2. The differences between group 1 and group 3 were highly significant at 2.4×10^{-3} and 4.8×10^{-3} molar solution, ($p <0.01$). The differences between group 2 and group 3 were highly significant at 0.64×10^{-3} ($p <0.01$) very highly significant at 1.2×10^{-3} ($p < 0\ 001$), 2.4×10^{-3} ($p <0.001$), and at 4.8×10^{-3} molar solution, ($p <0.001$) as shown in figure-1.

Responses to nor-adrenaline of vas deferens from control, group 2 and group 3 of rats: Nor-adrenaline dose-response curve of group 3 vas deferens preparations showed a significantly elevated maximal response from that of control and group 2 preparations. The elevated maximal responses were associated with no such difference at the lower part but with greater significance at the upper of the concentration-response curve. The maximal responses were (0.762 ± 0.073) for group 3, (0.435 ± 0.074) for the control group and

(0.265±0.062) for group 2. The differences between group 1 and group 3 were very highly significant at 0.64×10^{-3} ($p < 0.01$) significant at 1.2×10^{-3} ($p < 0.05$), very highly significant at 2.4×10^{-3} ($p < 0.001$) and highly significant at 4.8×10^{-3} molar solution, ($p < 0.01$). The differences between group 2 and group 3 were very highly significant at 0.64×10^{-3} ($p < 0.001$), 1.2×10^{-3} ($p < 0.001$), 2.4×10^{-3} ($p < 0.001$) and highly significant at 4.8×10^{-3} molar solution, ($p < 0.01$) as shown in figure-2.

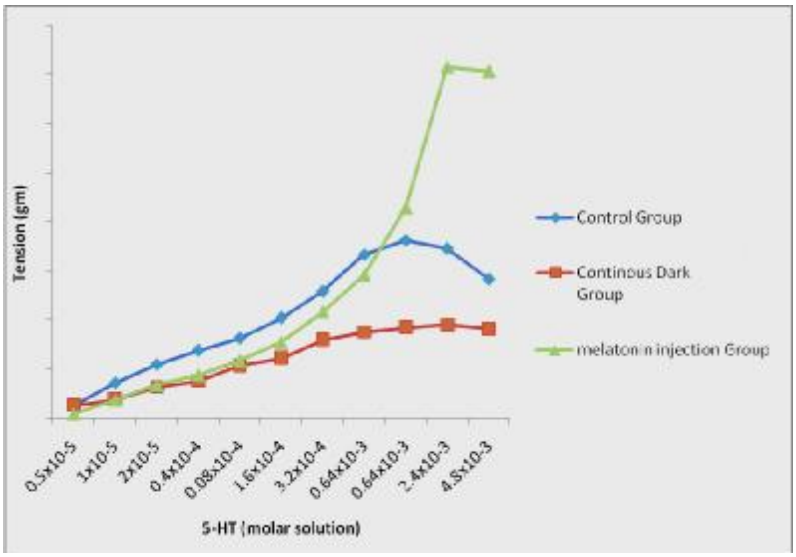

Figure 1: Response to 5-HT of vas deferens from control, continuous dark 4 weeks and continuous dark 2 weeks + melatonin injection groups.

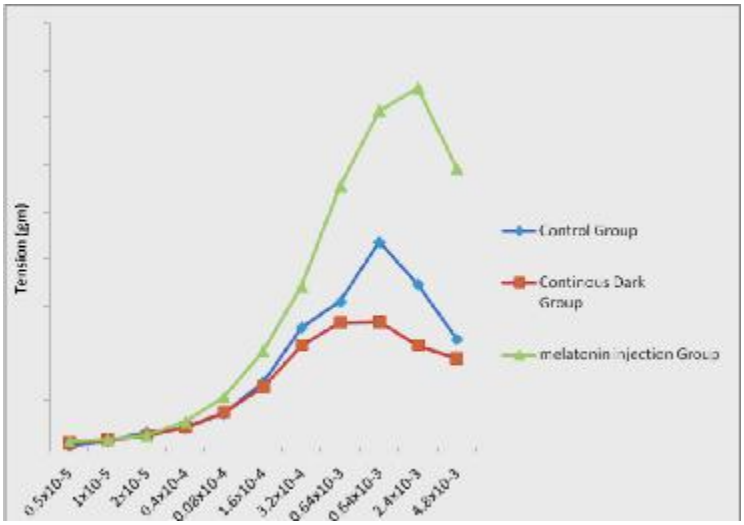

Figure-2: Response to nor-adrenaline of vas deferens from control, continuous dark 4 weeks and continuous dark 2 weeks + melatonin injection groups.

Discussion

Melatonin is the major secretory product of the pineal gland, playing an integral role in numerous metabolic, physiological and behavioral processes [8, 9].

This study aimed to investigate the possible effects of environmental changes as (continuous darkness) on the reactivity of the vasa deferentia smooth muscle in rats of similar age groups, The results showed that changes in the rhythm of the photo period have considerable effects on the reactivity of the vasa deferentia smooth muscle from rats to applied nor-adrenaline and 5-HT. two weeks of exposure to continuous darkness with melatonin treatment seemed to effect apparently the reactivity of the vasa deferentia smooth muscle to 5-HT and nor-adrenaline. The differences were significantly manifested especially at high doses between this group and both of the control and continuous dark 4 weeks groups. Vasa deferentia from melatonin animals responded to 5-HT with a greater maximal response, it was nearly twice that of control and nearly four times that of the continuous dark 4 weeks animals. Similarly, responses to nor-adrenaline were also greater maximal responses in the vasa deferentia from melatonin rats copared with those from contol and continuous dark 4 weeks animals. The maximal responses were nearly doubled compared with those from control animals and about 3 times as compared with those from continuous dark 4 weeks animals. This increased muscular reactivity can be explained by both direct and indirect action of melatonin.

The direct action of melatonin might involve cGMP (cyclic guanosine 3, 5-monophosphate) and prostaglandins.

Several studies suggest that cGMP may be a mediator of melatonin's CNS actions and a general effect of melatonin may be an increase in c-GMP in target tissues by increasing the activity of guanylate cyclase[10, 11].

Cyclic-GMP-mediated mechanisms may be involved in the effects of melatonin on testes, somniferous tubule contractility (gonads and accessory sex organs) and beta-adrenergic receptor sensitivity[1, 12].

The indirect action of melatonin may be caused by the release of nor-adrenaline and adenosine triphosphate from sympathetic nerve terminals in response to melatonin[13].

The other possible explanation for the stimulatory effect of melatonin on the contractility of the vas deferens smooth muscle may involve new receptor synthesis (receptor up-regulation mechanism). Since new protein synthesis is required for the synthesis of new receptors, and as melatonin enhance protein synthesis in the rat hypothalamus and cerebral cortex[1.2]; it may increase muscular reactivity through new receptor synthesis (receptor up-regulation), a possibility which needs further elucidation.

On the other hand, vasa deferentia preparations from continuous dark 4 weeks group of rats exhibited a reduced reactivity with a significantly lower

maximal response to 5-HT than those from control rats. It was found that the significantly elevated melatonin level after exposure to continuous darkness will stimulate the release of nor-adrenaline sympathetic nerve terminals[13].

In addition to the significant release of catecholamine after exposure to a prolonged stress (4 weeks of continuous darkness). This may depletes adrenergic nerves of the stored transmitter (nor-adrenaline). So that there is less transmitter available for release and since the contractile effect of 5-HT on the rat isolated vas deferens is prevailingly due to the release of nor-adrenaline (indirect action of 5-HT) [14], it may results in part in a reduced reactivity of vasa deferentia smooth muscle from continuous dark 4 weeks rats to 5-HT compared with that of control animals. The other possible explanation for this reduced reactivity may involve the action of melatonin on protein synthesis [15] and new receptor synthesis. Melatonin may not induce the synthesis of new receptors that are specific to mediate the effect of 5-HT on smooth muscle contractility of the rat vas deferens.

Therefore, the inhibitory effect of stress and receptor down-regulation will be more obvious resulting in a decreased reactivity to exogenous 5-HT compared with that of control animals.

More work is needed to explain further the effect of prolonged and/or acute administration using various concentrations of melatonin and different exposure time.

Conclusion

The present study proved experimentally that the vasa deferentia preparations from melatonin group responded with increased reactivity to nor-adrenaline and 5-HT when compared with those from 4 weeks darkness and control groups. The raised reactivity may be attributed to direct action of melatonin on vas deferens, an indirect action of the neurohormone via nor-adrenaline and ATP released from sympathetic nerve terminals, may be related to new receptor synthesis, or may be explained by all of these possibilities together.

In this study, the observed findings represent an attempt to explore the obscured changes which occur in the different body organs and tissues in response to the environmental changes and after melatonin treatment, therefore, deserve further studies in future. They have important implications both at the experimental and clinical level (persons who work in dark environment as miners, divers etc.)

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