The Effect of Continuous Darkness and Continuous Light on the Reactivity of Smooth Muscles to Drugs in the Rat Vas Deferens

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Abstract

Background: The vasoconstricting agents: nor-adrenaline and 5- hydroxytryptamine (5-HT) have a stimulant action on smooth muscle contractility of the rat vas deferens.

Objective: This study aimed to investigate the effect of exposure to continuous darkness and continuous light on the contractility of the vasa deferntia smooth muscles from rats to applied nor-adrenaline and 5-HT.

Method: Male albino wistar rats were divided into 3 experimental groups. Group 1: Control animals, were exposed to the ordinary photoperiod each day. Group 2: Rats were kept in a dark room. Group 3: In a room under a bright artificial light.

All animals were killed after 4 weeks.

Results: Vasa deferentia preparations from continuous dark group of rats exhibited a reduced reactivity with a significant lower maximal response to 5-HT than those from control rats. The maximal response in the control vasa deferentia preparations were nearly doubled compared with that of the continuous dark preparation where as they responded to exogenous nor-adrenaline with no significant difference from those of continuous dark animals. Vasa deferentia of continuous light animals responded with decreased reactivity and a smaller maximal response to both vasoconstrictors compared with that of control animals.

Conclusion: It is concluded that changes in the rhythm of the photoperiod have considerable effects on the reactivity of the vasa deferentia smooth muscle from rats to applied nor-adrenaline and 5-HT.

Key words: Vasa deferentia, nor-adrenaline, 5-HT, maximal response, melatonin.

Introduction

The rat vas deferens contains 5-hydroxytryptamine (5-HT) and it has been observed that the tissue concentrations of the amine are higher in regions nearer to the prostatic gland than the epididymis. Moreover, 5-HT seems to be synthesized and deaminated in the rat vas deferens. 5-HT causes contraction of smooth muscle, it has

both direct and indirect action on the rat isolated vas deferens. Direct effects are mediated by an interaction with specific 5-HT receptors on smooth muscle of the rat vas deferens. Indirect actions are prevailing due to the release of nor-adrenaline from adrenergic neurons.[1,2]

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 was more potent than that induced by 5-HT.[3]

In the present study we have investigated the effect of changing the environmental lighting conditions on the reactivity of the rat vasa deferentia smooth muscle to applied nor-adrenaline and 5-HT.

Materials And Methods

Drugs:

Serotonin creatinine sulphate (BDH) and nor-adrenaline bitartrate (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 a freshly prepared kreb's solution.

Solution: Experiments were performed by incubating the tissue in kreb's solution of the following composition (Quantities required for the preparation of 10 liters):

Nacl 69gm, 10% Kcl 35ml, 10% $MgSO_4$ $7H_2O$ 29ml, 10% KH_2 PO_4 16 ml, Glucose 20gm, $NaHCO_3$ 21gm, 10% $CaCl_2$ 25.2 ml.

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

Twenty male albino wistar rats, aged (4-5 weeks) weighing (76-92gm), 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.[4]

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

Group 2: Continuous dark animals, composed of 8 rats, was kept in a dark room for a period of 4 weeks.

Group 3: Continuous light animals, consisted of 6 rats, was maintained under a continuous bright artificial light for 4 weeks.

At the end of the predetermined period of exposure to light and darkness, the animals were anaesthetized with ether and killed by immediate exanguination.

The vasa deferentia were isolated, carefully cleared from all connective tissue surroundings *in vivo* before being cut between the prostatic and epidydimal attachment and then placed in a Petri dish filled with kreb's solution.

The vasa deferentia were 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 (95% O_2 and 5% CO_2 mixture) and warmed continuously at $37C^o$ .The tissue was left in the organ bath for 30 minutes to equilibrate with O.5gm tension on it and the mechanical activity was recorder isometrically by means of force-displacement transducer, type D-1, connected to a type 400 MP-2C physiograph recorder (George Washington LTD, A Searle CO.).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 vasa deferentia preparations and left in contact with the tissue for one minute, then the tissue was washed three times at 30 second intervals.

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 accumulative 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 ($P < 0.05$).

Results

1. continuous dark group:

A- Responses to 5-HT:

Vasa deferentia preparation from this group showed a reduced reactivity with a significant lower maximal response to 5-HT than those from control rats. The maximal response to 5-HT in the control vasa deferentia preparation was nearly doubled compared with that of the continuous dark preparation. The maximal responses ($\pm$ S.E) were (0.095 ± 0.026) for the continuous dark, as against ($0.181 \pm$

0.021) for the control vasa deferentia. The differences between group 1 and group 2 were significant at 3.2×10^{-4} ($P < 0.05$) highly significant at 0.64×10^{-3} ($P < 0.01$), 1.2×10^{-3} ($P < 0.01$) and significant at 2.4×10^{-3} molar solution, ($P < 0.05$)(**Table – 1**).

B- Responses to nor-adrenaline:

Vasa deferentia preparations from continuous dark group responded to exogenous nor-adrenaline with non significant difference from those of control animals (**Table-2**).

2.Continuous light group:

A- Responses to 5-HT:

Vasa deferentia from continuous light group responded with decreased reactivity and a smaller maximal response to exogenous 5-HT compared with that of control animals. The maximal responses were (0.091 ± 0.016) at 2.4×10^{-3} and (0.091 ± 0.023) and at 4.8×10^{-3} for the continuous light, as against (0.181 ± 0.021) for control vasa deferentia. The differences between group 1 and group 3 were significant at 0.8×10^{-4} ($P < 0.05$), 1.6×10^{-4} ($P < 0.02$), 3.2×10^{-4} ($P < 0.05$), very highly significant at 0.64×10^{-3} ($P < 0.001$) 1.2×10^{-3} ($P < 0.001$) and highly significant at 2.4×10^{-3} molar solution, ($P < 0.01$) (**Table-3**).

B- Responses to nor-adrenaline :

Vasa deferentia of continuous light animals responded with decreased reactivity and lower maximal responses to exogenous nor-adrenaline compared with those from control animals. The maximal responses were (0.258 ± 0.062) for the continuous light and (0.435 ± 0.074) for the control vasa defferentia. The differences between group 1 and group 3 were highly significant at 3.2×10^{-4} ($P < 0.01$), significant at 0.64×10^{-3} ($P < 0.02$) and at 1.2×10^{-3} molar solution, ($P< 0.05$) (**Table-4**).

Discussion:

Various kinds of environmental and psychological stress: light, darkness, cold, noise, emotional reactions of fear, anxiety and even smells and cage crowding, cause immediate and significant release of nor-adrenaline and adrenaline but had only a slight effect on the release of dopamine, the plasma concentration of nor-adrenaline and adrenaline were 5,11 folds higher than the basal values respectively.[5]

The initial response to stress involves primarily stimulation of the adrenal medullary system and with prolongation of the stress, the peripheral sympathetic system is

increasingly activated. Thereby, sympathoadrenal activity is significantly increased by stress stimuli, and the plasma levels of catecholamines reflect the degree of this activity.[6]

Stress also stimulate the synthesis and secretion of ACTH and corticosterone.The stress-induced released ACTH increases via corticosterone the activity of adrenal catecholamines – synthesizing enzymes[7,8] and subsequently cause the release of catecholamines to the systemic circulation. This prolonged high concentrations of the endogenously released agonists cause a reduction in the number of their plasma membrane receptors available for activation (receptor down – regulation) ,and consequently reduction in receptor affinity and sensitivity to the released catecholamines.[9] Therefore, the vasa deferentia smooth muscle of continuous light animals responded to the exogenous vasoconstrictors with a smaller maximal responses than those from control animals. However, vasa deferentia preparations from continuous dark group of rats responded to exogenous 5-HT with a reduced reactivity compared with that of control animals whereas they responded to nor-adrenaline with no significant difference from those of control animals. Exposing the animals of this group to 4 weeks of continuous darkness may have resulted in a marked increase in the synthesis and secretion of melatonin.[10,11]

This high circulating melatonin level may cause sympathetic overactivity with the release of nor-adrenaline and adenosine triphosphate from sympathetic nerves terminals or it may induce new receptor synthesis with (receptor up-regulation) since it is involved in protein synthesis.[9,12,13]

This stimulatory effects of melatonin is antagonized by the inhibitory effect of stress (4 weeks of continuous darkness) and the stress induced release of catecholamines with(receptor down-regulation).As a result, these 2 opposite responses will abolish each other's effects with the resultant effect being no significant alteration in responses to exogenous nor-adrenaline of vasa deferentia preparations from the above-mentioned groups.

On the other hand, vasa deferentia preparations from continuous dark group exhibited a reduced reactivity with a significantly lower maximal responses to 5-HT than those from control rats. It was found that the elevated melatonin level after exposure to continuous darkness will stimulate the release of nor-adrenaline from sympathetic nerve terminals. In addition to the already mentioned significant release of catecholamine after exposure to a prolonged stress.[14]

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 prevailing due to the release of nor-adrenaline (indirect action of 5-HT), it may result in part in a reduced reactivity of vasa deferentia smooth muscle from continuous dark rats to 5-HT compared with that of control animals. The other possible explanation for this reduced reactivity is that 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 reduced reactivity to exogenous 5-HT compared with that of control animals.

Conclusion

The present study shows that there was a marked decrease in muscular reactivity to exogenous 5-HT and nor-adrenaline of vasa deferentia preparations from rats after exposure to continuous darkness and continuous light for 4 weeks as compared with those from control animals, apart from responses to nor-adrenaline of vasa deferentia preparations from continuous dark rats (there was no significant difference from those of control preparations). The reduced reactivity may be attributed to an increase in the endogenously released catecholamines followed by receptor dow-regulation phenomenon.

Tables

(Table-1)

Responses to 5-HT of both vasa deferentia (right + left) from rats of control and continuous dark groups

Groups		No. of Animals	Doses (molar solution)				Response in mm (Deflection)
			3.2×10^{-4}	0.64×10^{-3}	1.2×10^{-3}	2.4×10^{-3}	
1	Control Animals	6	2.58 ± o.35	3.33 ±0.39	3.62 ± 0.43	3.45 ±0.45	
2	Continuous Dark animals	8	* 1.59 ± 0.23	*** 1.75 ± 0.18	*** 1.84 ± 0.41	* 1.9 ± 0.53	

***P < 0.05 *** P < 0.01**

(Table -2)

Responses to nor-adernaline of both vasa deferentia (right + left) from rats of control and continuous dark groups

Groups		Number Of Animals	Doses (molar solution)					Response in mm (Deflection)
			1×10^{-5}	0.8×10^{-4}	1.6×10^{-4}	0.64×10^{-3}	4.8×10^{-3}	
1	Control Animals	6	0.25 ±0.11	1.41 ± 0.14	2.75 ± 0.47	6.16 ± 0.81	4.58±0.82	
2	Continuous Dark Animals	8	0. 28 ± 0.11	1.46 ± 0. 38	2.53 ± 0. 67	5.28 ± 1.1	3.75 ± 1.46	

(Table -3)

Responses to 5-HT of both vasa deferentia (right + left) from rats of control and continuous light groups

Groups		Number Of Animals	Doses (molar solution)						Response in mm (Deflection)
			1.6×10^{-4}	3.2×10^{-4}	0.64×10^{-3}	1.2×10^{-3}	2.4×10^{-3}	4.8×10^{-3}	
1	Control Animals	6	2.04 ± 0.38	2.58 ± 0.35	3.33 ± 0.39	3.62 ± 0.43	3.45±0.45	2.83 ± 0.53	
2	Continuous Light Animals	6	** 0.87 ± 0.2	1.45 ± 0. 22	**** 1.58 ± 0. 16	**** 1.75 ± 0.2	*** 1.83 ± 0.32	1.83 ± 0.47	

**** P < 0.01 *** P < 0.01 **** P < 0.001**

(Table -4)

Responses to nor-adrenaline of both vasa deferentia (right + left) from rats of control and continuous light groups

Groups		Number Of Animals	Doses (molar solution)					Response in mm (Deflection)
			0.8×10^{-4}	$1. 6 \times 10^{-4}$	3.2×10^{-4}	0.64×10^{-3}	1.2×10^{-3}	
1	Control Animals	6	1. 41 ± 0.14	2.75 ± 0.47	5.08 ± 0.84	6.16 ± 0.81	8.7±1.48	
2	Continuous light Animals	6	0.83 ± 0.2	1.7 ± 0. 38	*** 2.33 ± 0. 44	** 3 ± 0.55	* 4.75 ±1.02	

*** P < 0.05 ** P < 0.02 *** P < 0.01**

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