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Research Article

THE EFFECT OF ASCORBIC ACID AND PROPRANOLOL ON NORMAL SLEEP AND OPEN FIELD LOCOMOTOR ACTIVITY IN WISTAR RATS

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ABSTRACT

Background: Knowledge about the actual central nervous system properties of propranolol or ascorbic acid remains poorly understood. Information on the neural mechanism of the interactions of these compounds in the central nervous system is still very hazy and presently a subject riddled with speculations. This study was designed to investigate the possible effects of ascorbic acid on open field locomotor activity and normal sleep in healthy adult rats and evaluate how these correlate with those of propranolol. **MethodS**: Eighty healthy adult Wistar rats of both sexes were divided into two groups of 40 animals each group. One group received ascorbic acid treatment while the other group received propranolol, both of which were prepared into different doses, respectively. Each of the various doses of these compounds was administered separately for a period of 21 days in each experimental group, while the control animals received 0.3ml normal saline for the same period. The results were subjected to statistical analysis, using MicroCal Origin Statistical Software version 8.0, and the Student's T- test to compare the results and P values of ≤ 0.05) were regarded as statistically significant. **Results**: Behavioral activation with increased locomotor activity and wakefulness were observed in doses of ascorbic acid while the animals appeared behaviorally-sedated with clear sleep promoting effects in the wistar rats in doses of propranolol. **Conclusion:** It is possible from present results that dopaminergic and cholinergic neural mechanisms account for the gross behavioral activation rats. It is clearly suggestive that the modulating influence on sleep

INTRODUCTION

When the behavioural output in the animal changes in magnitude, it is generally believed that the change must have resulted from alterations in central nervous system activity. Workers have noted that an alteration in central nervous system activity is influenced by humoral mechanism or by intrinsic activity (Ruch and Patton, 1966). In one other observation, it was noted that chemical agents modify the levels of neural activity in the brain to induce behavioural change. It is a known fact that one avenue through which a chemical agent may act to modify the behavioural output in the animal is through the interactions with biogenic amines.

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Sleep is a behavioural state in the animal, and it involves complex interactions with all the neural systems that traditionally regulate sleep in the brain. One of the ways it is possible to study sleep in an animal is by the application of a short acting barbiturate to evaluate the Onset Time (sleep latency) and Duration (total sleep time) of the induced sleep in the animal. On the other hand, it is also possible to employ a known substance such as ascorbic acid or propranolol (both of which are speculated to have some modulating influences on sleep) to study the time course of the normal sleep in relation to a known drug-induced sleep in the animal.

Apart from the neurotransmitter systems that are implicated in the central regulatory mechanism of sleep and behaviour, a number of endogenous factors have also been linked with sleep regulatory functions in the brain. In addition, the preoptic and posterior hypothalamic areas are known to be deeply involved in

the central regulatory functions of sleep, and some homeostatic and circadian factors are now believed to interact, not only to determine the timing of sleep, but also its quality (Siegel, 2005). For example, the inhibitory neurotransmitter GABA and Galanin are reported to initiate sleep by inhibiting the wakefulness and arousal regions of the brain (Siegel *et al*., 2001). These transmitter systems are known to have their origin in the ventrolateral preoptic nucleus of anterior hypothalamus, a nucleus believe to initiate and can inhibit the awake-promoting regions of the brain (Siegel, 2005). Furthermore, it is possible to undertake an overall assessment of a state of anxiety in the animal by way of a monitoring device which keeps track of the open field locomotor activity. Locomotor activities may be expressed in many forms including such diverse motor activities as swimming, flying, walking, running, rearing and hopping etc. (McCrea and Rybak, 2008).

Ascorbic acid (vitamin C), a water-soluble micronutrient have been noted for multiple biological functions including a cofactor for the synthesis of a number of enzymes in post-translational hydroxylation of collagen (Aguirre, 2008), it is involved in hematopoiesis and for leukocyte formation and tyrosine metabolism (Padh, 1990). Ascorbic acid is also known to be highly concentrated in the brain (Sjostrand, 1970; Kuo *et al*., 1979), and exhibits preferential distribution in the central nervous system (Subramanian, 1977), but is not synthesized anywhere in the brain in man and other primates (Vitler *et al*., 1967). Propranolol, on the hand, has been classified as a non-selective beta blocker, lipid soluble and exhibits sodium channel blocking effects. Propranolol is known to cross the blood–brain barrier (Steenen *et al.,* 2015), thus, it is possible that it may exert some central nervous system properties in addition to its known peripheral activities. It was the aim of this study to evaluate some open field locomotor activities and possible modulating influences on the normal sleep of rat in varying but separate doses of ascorbic acid and propranolol in Wistar rats.

MATERIALS AND METHODS

Eighty healthy rats of both sexes weighing between 180 - 200gm were used in this study. The animals were kept in cages under normal laboratory conditions and ambient temperature. The rats were fed with standard normal feeds and clean water throughout the experiments. The animals were allowed a period of two weeks to acclimatize to the environment of the laboratory before the commencement of the experiments.

The rats were organized into two groups of 40 animals each group. One group received ascorbic acid treatment in separate single doses 2,4,8,16,32,64,128 and 256 mg/kg body weight respectively, while the other group of 40 rats received propranolol treatment, of these same doses. Each experimental Group of 40 animals was further subdivided into 8 groups of 5 rats each. A group of 5 animals were treated with one of the eight separate doses throughout the period of the experiments. An animal was observed separately in a transparent perspex cage (40cm x 40cm x 1520cm walls (Dubovicky *et al.,* 2004) for locomotor activity for a period of one hour each session. In this way the 40 animals of a group was tested in the various doses of ascorbic acid or propranolol, respectively.

Determination of drug doses and administration

A known quantity of drug was dissolved in a volume of normal saline, to obtain the stock solution from where the different doses (2,4,8,16,32,64,128 and 256 mg/kg body weight) were determined according to the expression: Vol. (ml) = D x W x 1/C, where D is the required dose in mg/kg, W is the weight of the animal in kilograms and C, the concentration of the stock solution in mg/ml. These various doses were determination from the ascorbic acid and propranolol stock solutions, respectively.

The dose was administered intraperitoneally (i/p), after which the animal was observed in a quiet room free from disturbances and with ambient temperature of 24 ^{o}C, for behavioral activities, for a period of one hour. All experiments were restricted to the same seasonal period and between 900 hrs – 1800 hrs each day so as to avoid behavioral changes associated with seasonal and circadian variations.

Experiment for each dose was repeated 5 times using a fresh animal each time. The control group of five animals received 0.3 ml physiological saline only, for the test of either locomotor activity or normal sleep. Locomotor activity was registered when the rat crosses any of the line grids on the floor of the cage. The number of line crossings was recorded using a tally counting method. In the sleep experiments, the rats were regarded as asleep when they failed to respond to a very low noise level, thus, the Onset time and Duration of the normal sleep were recorded using a stop clock.

All of the behavioural and sleep data from all the experiments were pooled and subjected to statistical analyses, employing the Micro Cal Origin Statistical analysis Software, Version 8.0, and the Students' t-test, and the results were presented as Mean ± SEM, and p values ($p \leq 0.05$) regarded as statistically significant.

RESULTS

Data for the influence of ascorbic acid and propranolol on normal sleep locomotor activity are presented.

Table 1: Mean values of locomotor activity (line crossing) and Normal sleep patterns in separately administered doses of Ascorbic acid and Propranolol (n= 5).

Dose (Mg/kg)	Pattern of Sleep				Mean Locomotor Activity (Line Crossing)	
	Duration of Sleep (Min)		Onset of Sleep (Min)			
	Ascorbic acid	Propranolol	Ascorbic acid	Propranolol	Ascorbic acid	Propranolol
Control	0.00 ± 0.00	0.00 ± 0.00	>60.00 ± 0.00	>60.00 ± 0.00	98.40 ± 2.159	98.40 ± 2.159
2	0.00 ± 0.00	18.00 ± 1.41*	>60.00 ± 0.00	38.00 ± 1.140*	92.60 ± 3.043	93.20 ± 3.929
4	0.00 ± 0.00	24.00 ± 1.67*	>60.00 ± 0.00	33.00 ± 1.140*	94.40 ± 3.473	43.40 ± 3.280*
8	0.00 ± 0.00	27.40 ± 2.25*	>60.00 ± 0.00	27.00 ± 0.837*	86.00 ± 1.789*	23.80 ± 1.985*
16	0.00 ± 0.00	35.00 ± 1.70*	>60.00 ± 0.00	21.00 ± 1.342*	112.8 ± 4.271*	20.40 ± 1.631*
32	0.00 ± 0.00	40.00 ± 1.41*	>60.00 ± 0.00	18.00 ± 0.707*	127.2 ± 3.121*	10.40 ± 1.166*
64	7.60 ± 0.40*	51.60 ± 1.03*	50.40 ± 1.03*	9.000 ± 0.707*	131.2 ± 4.923*	2.000 ± 0.633*
128	5.20 ± 0.37*	0.00 ± 0.00	54.40 ± 0.68*	>60.00 ± 0.00	49.80 ± 1.985*	0.000 ± 0.000*
256	1.00 ± 0.37	0.00 ± 0.00	60.00 ± 0.00*	>60.00 ± 0.00	40.40 ± 2.015*	0.000 ± 0.000*

*Significant values (*P ≤0.05, **P ≤0.01, ***P ≤0.001) are Mean±SEM compared to control, (n= 5)

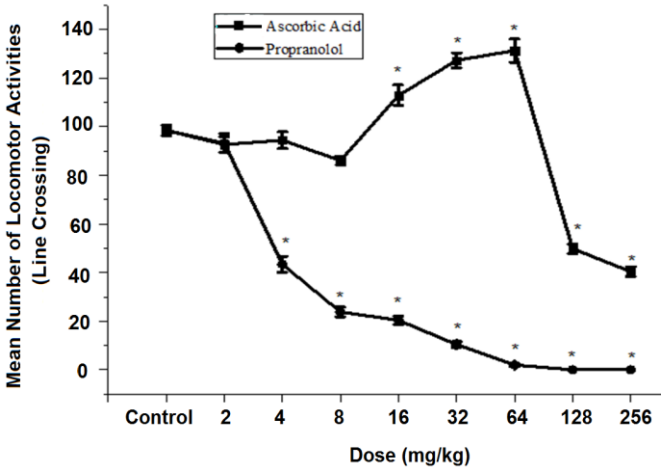

Fig.1: Mean values of locomotor activity (line crossing) in separate doses of Ascorbic acid and Propranolol in Wistar rats. *Significant values (*P ≤0.05, **P ≤0.01, ***P ≤0.001) are Mean±SEM compared to control (n= 5).

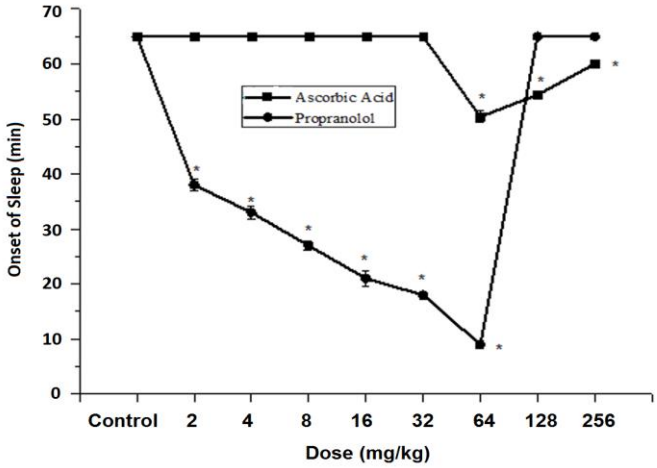

Fig. 2: Mean Onset time (min) of Sleep in varying doses of ascorbic acid and propranolol, respectively, in Wistar rats. *Significant values (*P ≤0.05, **P ≤0.01, ***P ≤0.001) are Mean ± SEM, compared to control, (n= 5). NB: Values greater than sixty (60) minutes indicate wakefulness.

DISCUSSION

The computed values of the behavioral data in this study indicate that the animals were generally behaviorally activated ($p \leq 0.05$) at the higher dose range of ascorbic acid (16-256mg/kg. Thus, the behavioural data in Table1 clearly indicate that ascorbic acid probably exhibits excitatory effects of on locomotor activity in wistar rats (please see also Figure1). On the other hand, values of the statistical data in Table1 revealed there were behaviorally depressive effects ($p \leq 0.05$) in the animals after their treatment with doses of propranolol (see Figure 1). Thus, this later observation in doses of propranolol treatment in this study is noted as largely in agreement with earlier reports that noted suppressive effects on locomotor activity in mice treated with propranolol (Eric *et al.*, 1995; Huaying *et al.*, 2011). Furthermore, the increase in open field locomotor activity observed in doses of ascorbic acid in this study is also noted as consistent with earlier reports of gross behavioral activation in rats following treatments with ascorbic acid (Abbasnejad and Shahsevari 2013), behavioral excitatory effects in low doses of administered ascorbic acid in Wistar rats (Ezenwanne and Anuka, 1991). The observation in the experiments with ascorbic acid in the present study also corresponds well with an earlier report which noted sedative influences (depressive

effects) in Wistar rats as the result of deficiency of ascorbic acid (Lyhs, 1961).

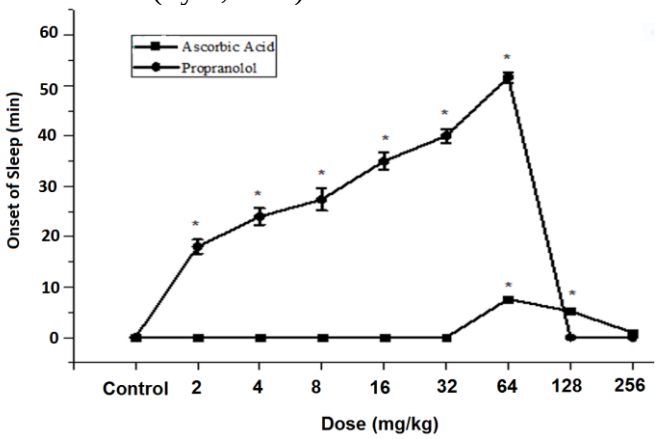

Fig. 3: Mean values of Sleep duration in separate doses of Ascorbic acid and Propranolol in Wistar rats. *Significant values (*P ≤0.05, **P ≤0.01, ***P ≤0.001) are Mean±SEM compared to control (n= 5).

The observation in open field locomotor activity in this study is noted as further proof of possible central nervous system excitatory property of ascorbic acid. Essentially, this is regarded as a clear demonstration which supports the notion or research proposals that ascorbic acid probably exhibits some clearly definable central nervous system activity of suppressive influence on central mechanism of sleep. The sleep study data also showed that the animals tended to stay longer time in sleep (sleep duration) after they were treated with propranolol, as opposed to the animals showing the greater tendency to remain longer in the state of wakefulness after treatment with ascorbic acid (please see Figure 3). The decreased Onset Time of sleep ($p \leq 0.05$) in doses of propranolol, as compared to the longer sleep Onset Time ($p \leq 0.05$) in doses of ascorbic acid in this study, can be noted as additional evidence which demonstrated that ascorbic acid and propranolol may exhibit mutually opposing properties in the central nervous system (Figure 2).

A number of earlier workers reported increases in sleeping time in rats and mice after treatments with doses of propranolol (Laverty and Taylor 1968), and there were also reports of increase in wakening time from sleep in humans after treatment with propranolol (Taşkın *et al*., 2015). There was also the proposal that sedative effect in the brain is generally linked to the GABA neural mechanism. In this study, sleep promoting influences of propranolol was clearly revealed within all the experimental animals. In the light of these reports and in view of the present observations, it is possible that the sleep promoting effects of propranolol in this study was mediated through GABA or Galanin neural mechanism. In a sleep model, some workers proposed the presence of a factor for sleep which is believed to use the inhibitory neurotransmitter GABA and Galanin to initiate sleep by inhibiting the arousal regions of the brain (Siegel *et al*., 2001; McDonald, 2010). Nevertheless, there are also the reports in which workers proposed that sleep production processes and behavioural effects of the brain are generally mediated through cholinergic neural mechanism (Espana and Scammell, 2004). Furthermore, in earlier reports also some workers were of the opinion that the central mechanism of action of ascorbic acid in the brain is generally mediated through dopaminergic system (Van Rossam and Hurkmans, 1964). However, presently research findings or reports on the possible central mechanisms of action of ascorbic acid are still very scanty. Nevertheless, it is possible that the cholinergic neural mechanism can account for the gross behavioral activation of ascorbic acid reflected in the increase in open field locomotor activity, observed in this study.

In view of the observations in this study, and in the light of similar reports by earlier workers, it is possible that the cholinergic and dopaminergic neural mechanisms. It is hoped that future studies will further elucidate the specific neural mechanisms implicated in specific behavioral parameter in the central nervous system.

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