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Effects of Melatonin on Startle Reflex in Rat

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DATTA, P. C., F. K. HOEHLER AND C. A. SANDMAN. *Effects of melatonin on startle reflex in rat.* PEPTIDES 2: Suppl. 1, 155–160, 1981.—In two experiments the effects of melatonin were investigated on the motor, cardiac and emotional components of the startle reflex induced by an intense acoustic stimulus. Melatonin inhibited the motor movements and significantly decreased the HR responses of rats pretested with these procedures. Melatonin decreased the HR responses to stimulation whereas the HR responses of the rats treated with the vehicle solution increased. Melatonin treatment also inhibited the startle-induced defecation compared with the vehicle injection. These findings suggest that melatonin exerts inhibitory effects on the CNS, on sensory reflex and on stress responses.

Melatonin Startle reflex Defecation Habituation Inhibition

THE inhibitory effect of melatonin on stress responses in human and infrahuman subjects has been well documented [1, 3, 4–6, 9, 20, 23, 24, 26]. Datta and King demonstrated that melatonin administered intraperitoneally (IP) leads to a decrease in defecation in passive avoidance (PA) tasks [4] and in novel environments [5]. Rats treated with melatonin and exposed daily to test box show a significant habituation of plasma 11-hydroxycorticosterone (11-OHCS) levels along with an inhibition of defecation response [5]. Concomitant with the inhibitory effect on stress responses (defecation and plasma 11-OHCS) treatment with melatonin also leads to an increase in whole brain dopamine (DA) [5]. Datta and King [5,6] have suggested that melatonin may inhibit or habituate stress responses via action on either the serotonergic [2] or the dopaminergic systems [5]. This modulation of the inhibitory neurotransmitters by melatonin may account for behavioral effects observed with other behavioral paradigms. Evidence of the putative relationship between melatonin and behavior is equivocal. Some reports indicate that melatonin exerts inhibitory effects on motoric behavior [12, 25, 30, 31], whereas other reports indicate that melatonin has no inhibiting influence on motor behavior [4, 5, 17]. Perhaps the aspect of motoric activity which should be investigated after treatment with melatonin is the startle reflex (SR), elicited by an intense stimulus. Since the SR may be generated below the midbrain (probably in the nucleus reticularis pontis caudalis [21]) and melatonin increases the concentration of serotonin (5-HT) in the midbrain and the hypothalamus [2], and that melatonin increases brain gamma aminobutyric acid (GABA) [1] and increases brain DA over 5 days of treatment [5], it can be expected that an intense stimulus-induced SR may be inhibited or habituated significantly by melatonin. A significant habituation of motoric startle response was previously observed in animals treated with clonidine, an adrenergic agonist [10]. However, repetitive elicitation was also found to lead to habituation of the SR [10]. Marczynski, Yamaguchi, Ling and Grodzinska [22] were the first to observe that bilateral administration of melatonin in the preoptic region decreased or inhibited the reactivity of the treated cats to acoustic stimuli (which were effective to startle them in their non-drugged condition). They further observed that the heart rate (HR) of these animals decreased by about 20–30% of the mean initial resting values during experimentation. Two experiments were conducted to elaborate the findings of Marczynski *et al.* [22] and to verify the inhibitory effects of melatonin on the motor and cardiac components of the SR induced by an intense acoustic stimulus.

EXPERIMENT 1

Melatonin has been shown to be a potent inhibitor of motoric behavior in mammal and avian subjects [12, 22, 25, 31, 32]. However, Datta and King [4,5] and Kastin *et al.* [17] could not establish an inhibitory effect of melatonin on activity in their behavioral studies. Yet melatonin has been

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reported to decrease responsiveness to auditory (sensory) stimuli [22] and to reduce sensory reflex epilepsy in cats [11]. Anton-Tay *et al.* [3] also reported that melatonin treatment slowed down the cortical and sub-cortical electrical activity in epileptic subjects followed by sleep induction; paroxystic activity was also markedly depressed. Hence melatonin may affect the threshold of the startle reflex (SR) generated in the lower part of the midbrain [21] and may reduce the motor and cardiac components of the SR generated by an intense acoustic stimulus. In this experiment the effect of melatonin was investigated on the decrease or habituation of the motor and HR startle responses in rats exposed to the procedures.

METHOD

Animals

The animals were 12 male Sprague-Dawley Albino rats, 130–140 days old (450–550 g) at the beginning of the experimentation. These animals were pre-treated with α-MSH, β-endorphin or its vehicle solution on 3 separate days in a counterbalanced order, with 3 day washout periods, and were presented with 30 acoustic stimuli on each treatment day [13]. The animals were randomly assigned to melatonin treatment or its vehicle injection, with 6 rats per treatment per day. A 5 day rest period was allowed to the rats prior to the beginning of experimentation. The animals had ad lib access to food and water and were housed 3 in each cage, in an air-conditioned holding room (approximately 70°F).

Apparatus

The apparatus consisted of a blue Plexiglas cylinder (17.5×17.5×42 cm) with a platform sensitive to the movements of the rat. A chest strap fitted with electrodes permitted measurement of the EKG and connected to a Grass Model 7 Polygraph. Motoric responses were recorded by the electrically active floor of the cylinder which also connected to the polygraph. The animals were placed in the cylinder and then in an acoustic testing chamber. The acoustic stimulus (i.e., a 118 dB noise of 1000 Hz square wave lasting for 60 msec) was presented in the testing chamber (115×100×134 cm) and controlled by a logic panel and a sound generator.

Procedure

Injection procedure. Each rat received an IP injection of either melatonin (250 μg/rat in 0.5 ml) or its vehicle solution (0.9% NaCl + 0.01 M acetic acid + 2% ethanol in 0.5 ml) on alternative days in a counterbalanced order (following a reversal design), using each rat as its own control, e.g., day 1 melatonin, day 2 rest, day 3 vehicle, day 4 rest, day 5 melatonin, and so on until each rat received 2 melatonin and 2 vehicle injections.

Behavioral procedure. The animals were brought to the testing room 60 min after injection. The rat was slowly inserted into the cylinder, which the animal did voluntarily and easily. As the rat accommodated to the cylinder, and artifact-free EKG tracings appeared, the cylinder with the animal was placed in the testing chamber. After 3–5 min 30 stimuli were presented (118 dB of 1000 Hz square wave for 60 msec) with an intertrial interval (ITI) of 15 sec.

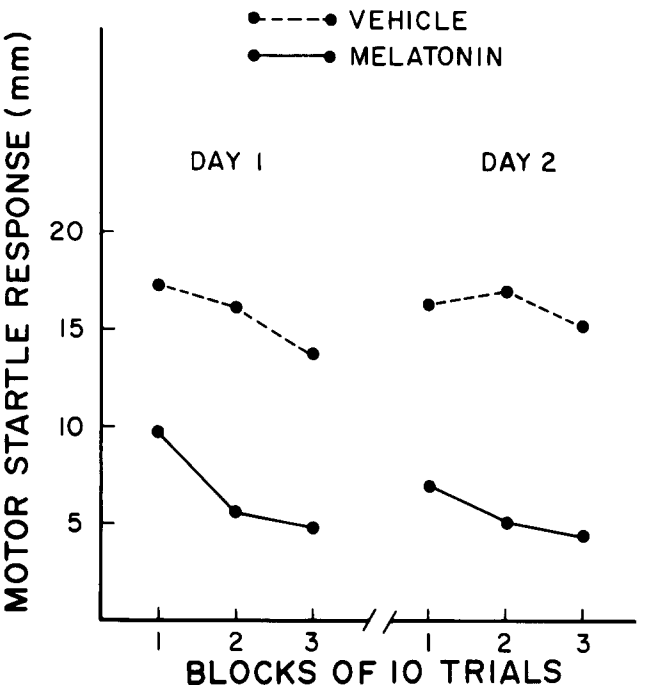

FIG. 1. The mean values of motor startle responses (mm) for the melatonin and vehicle treated rats over days 1 and 2.

RESULTS AND DISCUSSION

The motor startle responses of rats tested with melatonin or vehicle are shown in Fig. 1. Results of a 3-way ANOVA showed that the drug effect was highly significant, $F(1,11)=92.33$, $p<0.001$, indicating a significant inhibitory effect of melatonin on the motor component of the startle reflex. The habituation of the motor response for the two groups over 3 blocks of 10 trials was also significant, $F(2,22)=6.15$, $p<0.01$, suggesting that treatment with melatonin did not influence habituation.

The baseline cardiac responses (beats per minute (BPM), 1 sec prior to stimulation) for the melatonin and the vehicle treated rats over days 1 and 2 are shown in Fig. 2. The habituation of the baseline HR over blocks of trials was significant, $F(2,22)=5.58$, $p<0.05$, indicating a significant decrease of HR over trials for both the melatonin and the vehicle treated rats. As can be seen in Fig. 2 the baseline HR of the vehicle group was lower on day 1, but this difference disappeared on day 2.

Figure 3 shows the means of HR at 5 sec (the difference between baseline HR and HR at 5 sec post stimulation) and the means of HR increase (cyclic maximum) (the difference between the baseline HR and the largest HR in 5 sec) of the melatonin and the vehicle treated rats over days 1 and 2. The effect of melatonin on the 5 sec measure was highly significant, $F(1,11)=69.00$, $p<0.001$, indicating that melatonin exerts dramatic effects on cardiovascular responses. The decrease of HR due to melatonin at 5 sec post-stimulation was so significant it fell below the baseline HR (see Fig. 3).

The effect of melatonin on HR increases was also significant, $F(1,11)=6.52$, $p<0.05$. This suggests that the overall effect of melatonin was to suppress cardiovascular responses

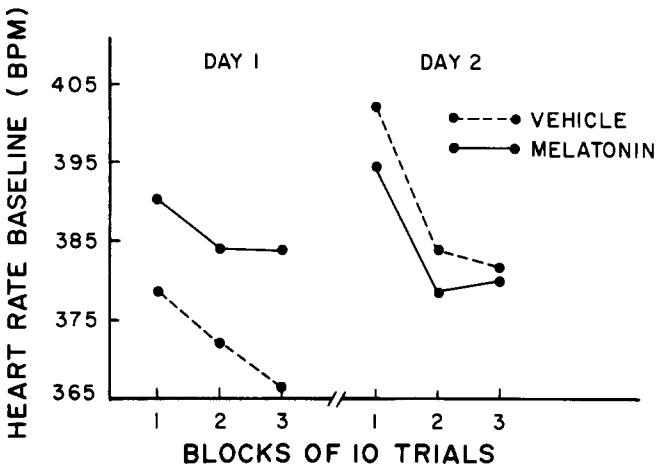

FIG. 2. The means of baseline HR responses (1 sec pre-stimulation) for the melatonin and the vehicle treated rats over days 1 and 2.

in a condition likely to elicit such response. The drugs × days interactions effect was significant, $F(1,11)=8.37$, $p<0.05$, suggesting that the effect of melatonin on the HR increase was primarily observed only in the first session. The decrease of the HR component of the SR due to melatonin treatment (compared with its vehicle injection) was conspicuous. These findings support the observations of Marczynski *et al.* [22] that melatonin decreases post stimulation HR. The significant habituation of the baseline HR also support the observation of Davis *et al.* [10] that repetition of a stimulus leads to a habituation of the startle reflex responses. Melatonin, while exerting a strong inhibitory effect on the motor and cardiac startle responses, does not influence the process of habituation.

EXPERIMENT 2

A second experiment with naive rats was conducted to verify the effects observed in Experiment 1 with experienced rats. Further, the inhibitory effect of melatonin on stress-induced defecation reported by Datta and King [4,5] was extended using the startle situation as a stressor (novelty + loud noise). Both novelty and loud acoustic noise are capable of inducing defecation responses in rats [5,15]. Experiment 2 was, therefore, conducted to investigate the effects of melatonin on (1) inhibition of motor startle responses, (2) habituation of baseline HR, (3) abatement of increase of post stimulation HR, (4) decrease of HR at 5 sec after stimulation, and (5) inhibition of defecation induced by the startle situation.

METHOD

Animals

The animals used in this experiment were 12 naive male Sprague-Dawley Albino rats, 110–120 days old (350–450 g) at the time of testing. The animals were randomly assigned to the two orders of treatment groups. Six rats received melatonin on day 1 and six rats received the vehicle on day 1. The rats were housed 3 in a cage in the holding room (70°F) and had ad lib access to food and water.

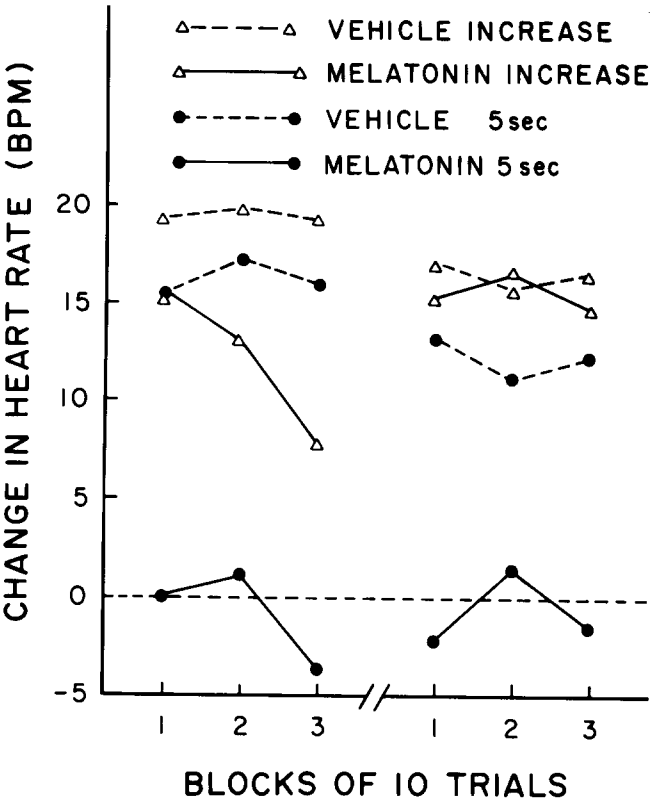

FIG. 3. The means of HR 5 sec (the difference between baseline HR and the HR at 5 sec) and the means of HR increase (the difference between baseline HR and the largest HR in 5 sec post stimulation) for the melatonin and vehicle treated rats over days 1 and 2.

Apparatus

The apparatus and accessories used in this experiment were the same as used in Experiment 1.

Procedure

Injection procedure. The injection procedure was the same as in Experiment 1 except that in this experiment each rat received 4 melatonin and 4 vehicle injections on separate days with a rest day between each treatment.

Behavioral procedure. Prior to the beginning of experimentation the rats were handled for 3 min each day for 7 days [5]. The procedures for recording the motor and cardiac responses to the 30 acoustic stimulus presentations were the same as in Experiment 1. In this experiment the additional behavioral procedure was the recording of bolus frequencies observed during 20 min of the experimental session. The boluses were recorded from the time the rat was placed in the cylinder until it was taken out of the cylinder. After each rat was taken to its home cage, the boluses were counted and the cylinder was cleaned and dried.

RESULTS AND DISCUSSION

The mean motor startle responses for the rats treated with melatonin or vehicle over days 1–4 are shown in Fig. 4.

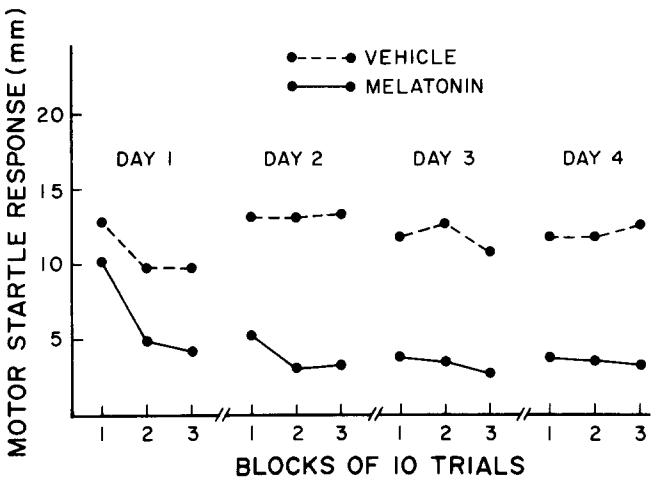

FIG. 4. The means of motor startle responses (mm) for the melatonin and the vehicle injected rats over days 1–4.

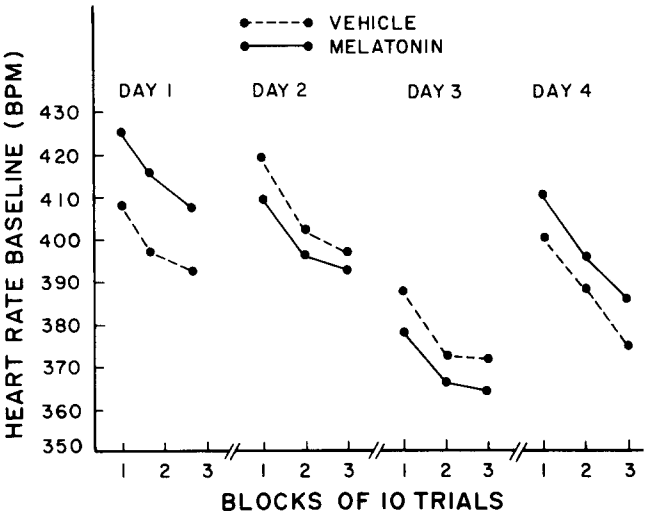

FIG. 5. The means of baseline HR (1 sec pre-stimulation) for the melatonin and the vehicle treated rats over days 1–4.

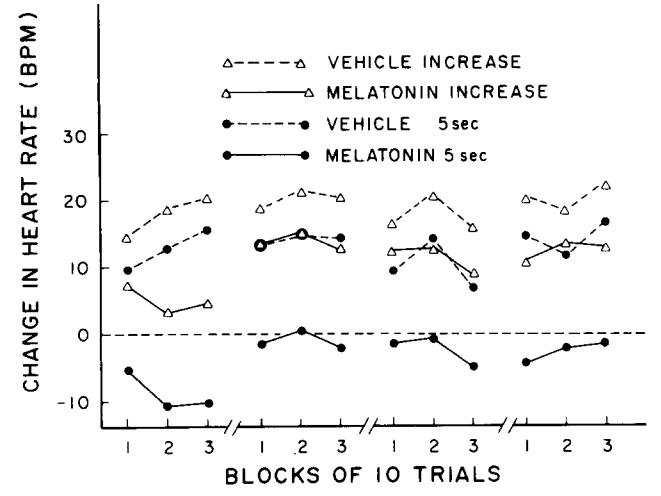

FIG. 6. The means of HR increase (baseline HR–HR at 5 sec post-stimulation) and the means of HR increase (baseline HR–maximum HR in 5 sec post-stimulation) for the melatonin and the vehicle injected rats over days 1–4.

Results from a 3-way ANOVA were consistent with the findings for Experiment 1. Melatonin signifiantly reduced the motor startle responses over the days of treatment, $F(1,11)=46.61$, $p<0.001$. The habituation effect within days was also significant, $F(2,22)=6.56$, $p<0.01$, further supporting observations in Experiment 1. However, habituation of the motor startle was not the same for each day, and hence the days×habituation interaction was significant, $F(6,66)=4.65$, $p<0.01$ (see Fig. 4). Indeed, very little habituation across days was observed.

The means for baseline HR of the melatonin and vehicle treated animals over days 1–4 are shown in Fig. 5. Analysis of the baseline HR data suggests that the HR differed between days of treatment (decreasing over days), $F(3,33)=20.73$, $p<0.001$, and that there was a consistent habituation of baseline HR over days, $F(2,22)=34.33$, $p<0.001$. These results indicate an habituation of the HR of the animals both over trials and days of exposure to the startle situation, and this habituation was free from the effect of melatonin.

The means of HR recorded at 5 sec (post stimulation) and the means of HR increases (based on the difference between the baseline HR and the maximum HR in 5 sec) for the melatonin and the vehicle treated rats over days 1–4 are shown in Fig. 6. The data reveal that compared with its vehicle injection melatonin significantly decreased HR at 5 sec after stimulation, $F(1,11)=114.12$, $p<0.001$. However, this decrease of HR at 5 sec was different for the 4 days over the 3 blocks of trials which resulted into a significant days × habituation interaction, $F(6,66)=3.02$, $p<0.05$. As can be seen in Fig. 6 and consistent with Experiment 1, the post-stimulation (at 5 sec) HR of the rats treated with melatonin fell below baseline HR. Analysis of the HR increase data showed that melatonin significantly abated the maximum rise of HR in 5 sec post stimulation, $F(1,11)=37.56$, $p<0.001$. The increase of HR over days and over trials was not different and the interactions with treatment were not significant. These results suggest that melatonin not only has an inhibitory effect on the motor startle responses but also exerts a significant inhibitory effect on the cardiac acceleratory component of the SR.

The means of the bolus frequencies for the melatonin and the vehicle treated rats over days 1–4 are shown in Fig. 7. Melatonin significantly inhibited the startle-induced defecation in rats, $F(1,11)=31.56$, $p<0.001$. This result supports earlier findings [5] of a significant inhibitory effect of melatonin on novelty-induced defecation. However, in the current experiment the habituation effect was not significant, which suggests a possible carrying over effect of melatonin on the startle-induced defecation, since each rat was treated with melatonin and its vehicle solution on alternative days, whereas Datta and King [5] injected the same rat either with

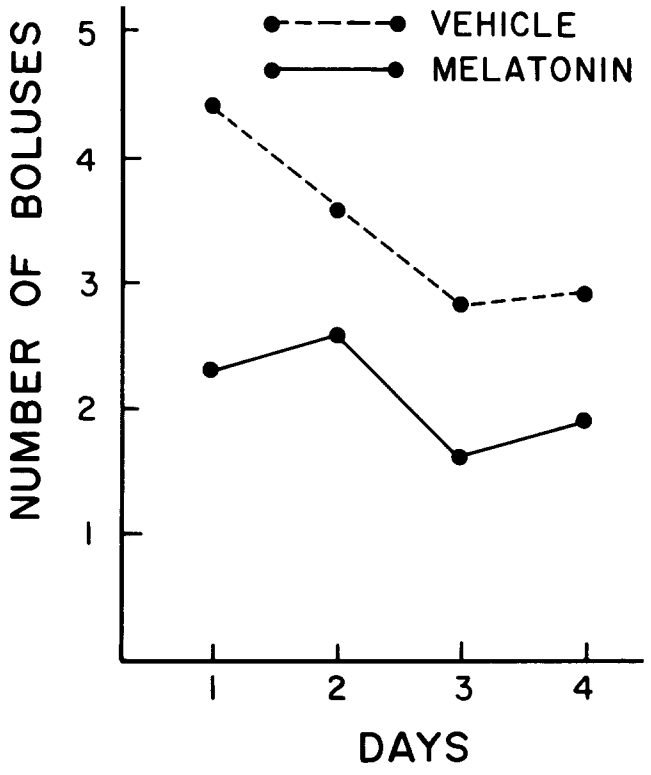

FIG. 7. The means of bolus frequencies for the melatonin and the vehicle treated rats over days 1–4.

melatonin or its vehicle solution on each day, and daily exposed the animals to the same environment.

Overall results of Experiment 2 support and replicate the findings of Experiment 1, and hence, it can be assumed that the inhibitory effects of melatonin on motor and cardiac components of the SR are free from the previous experience.

DISCUSSION

The overall results of Experiments 1 and 2 suggest that the motor movements and cardiac components of the startle reflex are strongly inhibited by melatonin treatment. In both the experiments the motor startle response inhibition by melatonin was highly significant ($p<0.001$), and melatonin proved to be a potent inhibitor of the dependent measures in both naive (Experiment 2) and pre-treated animals (Experiment 1). The present findings suggest that the effects of melatonin on the motor and cardiac components of the SR might have been washed out over days (e.g., on a vehicle injection day after 1 rest day). The rats treated with the vehicle solution did not seem to retain the inhibitory effect of melatonin on the motor and cardiac measures that they showed on a melatonin treatment day. It was observed by Anton-Tay *et al.* [3] that the effect of melatonin on the inhibition of electrical activity in the cortical and subcortical structures seemed to last about 5 hours after which the electrical activities returned to the normal level. Similarly, the rise in brain 5-HT and GABA after melatonin treatment remained high up to about 360 min after treatment and then habituated to the baseline [1,2]. Hence, the inhibitory effect of melatonin on the motor and cardiac responses may also be time dependent.

The decrease of HR to the baseline (or below the baseline) at the 5th sec after stimulation suggests a strong normalization (or habituation to the resting level) effect of melatonin on the cardiac responses in the presence of an arousing stimulus. The HR of the rats treated with the vehicle showed an increase after the onset of the stimulation and this increase was still maintained at 5 sec post stimulation (after which the HR decreased or habituated to the baseline), but the HR of the rats treated with melatonin did not show an equivalent increase over their base HR. Further, their HR habituated to the base level and fell below the base level at 5 sec post stimulation. This observed inhibitory effect on the cardiac component of the SR could be accomplished by melatonin in the following way: (a) Melatonin treatment leads to an increase in the concentrations of brain 5-HT, GABA and DA [1, 2, 5] which may exert inhibitory effects on the cardiac output of the SR. Melatonin also is considered as an inhibitory neurotransmitter by itself [5, 6, 9, 29]. (b) Melatonin decreases the electrical activity in the midbrain and in the subcortical structures which may be implicated in the generation of the startle reflex [3,21] and this effect subsequently may increase the threshold of sensory reflex for acoustic stimuli [11]. (c) Recently, Iramain and Owasoyo [14] have shown that melatonin injected sub-cutaneously (SC) (1.25 mg/kg), twice a day, for 6 days, led to an increase in the activities of choline acetyltransferase (chAT) in the hypothalamus, cerebral cortex, mesencephalon and hippocampus, and also led to an increased activity of acetylcholinesterase (AchE) in the hippocampus and cerebral cortex. The increase in chAT and AchE activities in the brain (which may indicate increased synthesis or functioning of acetylcholine) after melatonin treatment may suggest a parasympathetic control (restoration or conservation as opposed to acceleration or arousal) exerted by melatonin on the cardiac output in response to environmental stimulation. (d) Kastin and associates [18] observed that treatment with α-MSH increased HR of female subjects, but this effect of MSH on HR could not be established in subsequent studies [27,28]. Sandman *et al.* [28] reported an improved orienting response (OR characterized by decelerated HR) in human subjects after MSH/ACTH 4–10 treatment. The interaction between MSH and melatonin is well known [4, 9, 17, 19]. Melatonin releases pituitary MSH via inhibiting MIF-I in the hypothalamus [5,19] and the released MSH gets metabolized very quickly, i.e., in about 2 minutes [18]. The observed inhibitory effect of melatonin on the cardiac responses therefore may not be separate from the effect exerted by the endogenous levels of MSH or MIF-I which may slightly exacerbate the cardiac responses [13,16].

The observed inhibition of startle situation-induced defecation of the treated animals suggests that melatonin reduces the impact of stress as has been observed in animals exposed to passive avoidance [4,9] or to novelty [5]. However, the defecation of the vehicle injected rats also decreased over days though not significantly (see Fig. 7). It is interesting that a similar decrease of defecation was paralleled by a decrease of baseline HR over days 1–4 for the melatonin and the vehicle treated rats (Figs. 5 and 7), but only the decrease (habituation) of HR over days was significant. The significant habituation of the baseline HR and of motor startle responses (Experiment 2) supports the contention of Davis

et al. [10] that repetition of the acoustic stimulus leads to an habituation of the startle response over trails. The present study extends the findings to include the effect that HR habituation may also take place over days of exposure to the same, intense stimulus.

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