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Effects of MIF-I and Melatonin on Novelty-Induced Defecation and Associated Plasma 11-OHCS and Brain Catecholamines

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DATTA, P. C. AND M. G. KING. *Effects of MIF-I and melatonin on novelty-induced defecation and associated plasma 11-OHCS and brain catecholamines.* PHARMAC. BIOCHEM. BEHAV. **11**(2) 173–181, 1979.—In two experiments the effects were investigated of MSH-inhibiting factor-I (MIF-I) and of Melatonin on step-down latencies, defection, plasma 11-OHCS levels, whole brain DA and whole brain NE concentrations on Days 1, 3 and 5 of novelty exposure. Treatment with MIF-I led to a significant habituation of novelty-induced defecation over 5 days, whereas plasma 11-OHCS level was reduced only on Day 1. The concentrations of whole brain DA and whole brain NE also showed a significant increase over days of MIF-I and novelty treatment. Melatonin treatment, on the other hand, significantly inhibited novelty-induced defecation and reduced plasma 11-OHCS level on Day 5 of novelty exposure. Melatonin treatment led to a significant increase of whole brain DA in animals exposed to novelty for 5 days. Neither MIF-I nor Melatonin was found to significantly affect the step-down activity of treated animals. The overall results suggested a possible relationship between novelty-induced defecation and brain DA levels of MIF-I and Melatonin treated animals.

Melanocyte-stimulating hormone (MSH)-release inhibiting factor I Melatonin Step-down latency Novelty-induced defecation Plasma 11-hydroxycorticosterone (11-OHCS) Dopamine (DA) Norepinephrine (NE)

THE hypothalamus secretes a tripeptide, identified as Pro-Leu-Gly-NH_2 and referred to as melanocyte-stimulating hormone inhibiting factor-I (MIF-I), which inhibits specifically the secretion of MSH from the pituitary gland under several conditions [22, 23, 24, 27, 33, 40, 41]. It has also been shown that Melatonin, a pineal hormone, significantly depletes pituitary MSH in rats if injected intraperitoneally (IP) and that this effect of Melatonin disappears when the hypothalamus of such an animal is destroyed [22, 23, 24, 25, 27, 28]. It has been suggested by Taleisnik Tomatis and Celis [41] that the depleting effect of Melatonin on pituitary MSH is exerted through its effect on the serotonergic and catecholaminergic (probably dopaminergic) systems in the hypothalamus. Serotonin (5-HT) content of the rat brain is raised significantly within 1 hour after IP injection of Melatonin [2]. The paraventricular nuclei (PVN) of the hypothalamus which elaborate and secrete MIF-I are innervated by catecholaminergic neurons. The elevation of brain 5-HT after IP injection of Melatonin probably activates an inhibitory system which has been claimed to curtail the catecholaminergic afferent impulses to the PVN. This in turn suppresses MIF-I secretion and thus depletes pituitary MSH [41].

It is very likely that central catecholamines, especially dopamine (DA), are implicated in behavior consequent upon MIF-I or Melatonin administration. It has been shown that PLG potentiates certain behavioral effects of DA [35,37]. It has also been reported that both MIF-I and Melatonin alleviate Parkinson tremors in humans and in infrahuman preparations [3, 34, 35, 36, 37]. Clinical studies using MIF-I and Melatonin have shown that patients who have been injected with these neuropeptides report a feeling of well-being and elation [3,12]. Hence it is very likely that both MIF-I and Melatonin affect behavior by way of the central catecholaminergic system.

It has been reported that MIF-I treatment leads to a significant rise in brain DA in the striatum of intact rats [14] and in whole brain of both intact and hypophysectomized rats [23]. Others have reported null effects of MIF-I on DA concentration in the caudate nucleus or whole brain [37]. None of the above investigators has reported a significant effect of MIF-I on norepinephrine (NE) concentration in whole brain.

The similarity between MIF-I and Melatonin in their effects on Parkinson tremors and on mood, e.g., reported feelings of well-being, is probably mediated by an increased turnover of whole brain DA concentrations and not by increased 5-HT levels since Melatonin treatment increases 5-HT content of brain whereas MIF-I reduces 5-HT content in hypox rats [3,39]. In addition some investigators have reported that very small doses of DOPA, the immediate precursor of DA, and small doses of MIF-I potentiate the skin lightening effect of Melatonin [27].

It has been shown recently by Datta and King [10] that Melatonin treatment significantly inhibits the defecation response in animals undergoing passive avoidance (PA) learning. An opposite facilitatory effect of MSH on PA-induced

defecation [10] and on novelty-induced defecation was accompanied by a concomitant reduction in whole brain DA and whole brain NE concentrations [11]. It is predicted therefore that Melatonin treatment should inhibit novelty-induced defecation and concomitantly increase the brain concentrations of DA and NE. If brain CAs are implicated in the maintenance of novelty-induced defecation by MSH, it can be expected that MIF-I would inhibit novelty-induced defecation through increasing brain CA concentrations over days of treatment. The present study investigated the effects of MIF-I and of Melatonin on novelty-induced defecation and associated Novelty×Drug-induced changes in plasma corticosterone (11-OHCS) and whole brain CAs (NE and DA) over successive treatment days.

EXPERIMENT 1

It has been demonstrated by Datta and King [11] that administration of MSH leads to a significant reduction of whole brain NE and DA concentrations in animals exposed to a novel environment and sustains novelty-induced defecation over several days of treatment. In view of these findings the implication of the putative inhibitory neurotransmitters, NE and DA, is investigated in novelty-induced defecation is a function of whole brain CA concentrations, especially NE [11], MIF-I treatment may well lead to habituation of such novelty-induced defecation responses by increasing whole brain NE and whole brain DA. It has been reported earlier by Friedman *et al.* [14] that the whole brain DA level is significantly elevated after 4 days of treatment with MIF-I.

Since DA is the immediate precursor of NE it would be expected that MIF-I treatment will lead to an increase in both brain DA and NE concentrations over days. Such a rise in brain CAs, which have been claimed by several investigators [8, 15, 38] to be inhibitory neurotransmitters in the pituitary-adrenal axis, is also expected to lead to an inhibition of plasma 11-OHCS levels in MIF-I treated rats.

METHOD

Animals

The animals were 36 naive male Wistar rats, aged 90–100 days at the beginning of experimentation. They were randomly assigned to three treatment duration groups: Day 1, Day 3 and Day 5, with 12 rats per group. Rats in each treatment duration group were further randomly assigned, with six per group, to either MIF-I treatment or to the control injection. Animals in the three treatment duration groups were adapted to a 12:12 hr light:dark cycle for 4 weeks prior to the start of the experiment. They had ad lib access to food and water throughout the experiment.

Apparatus

The test box used as a novel environment in this experiment has been described in detail by Datta and King [10] who used the test box as a stepdown passive avoidance (PA) box. The box was made of Plexiglas with internal dimensions of 30×35×45 cm to the grid floor. In one corner of the box there was a 15×15 cm Plexiglas platform fixed 3.5 cm above the grid floor and attached to a corner wall. The platform was covered with masking tape to prevent animals from slipping and was balanced on a micro-switch which was tripped by the weight of the rat. The switch was wired to a minicomputer which operated a print-out timer and an electronic clock. Nine cm below the grid floor there was a metal tray covered by paper towelling. The test box was covered externally with opaque adhesive paper and the top was open. The test box and other equipment were housed in a light-proof, sound-lagged air-conditioned cubicle in which the temperature was maintained at 23 ± 1°C. The ambient illumination in the test box was 35 lx.

Procedure

Injection procedure. The rats in each experimental group received a daily IP injection of MIF-I (250 μg/rat in 0.25 ml) and in the control group an IP injection of 0.9% NaCl and 0.01 M acetic acid in 0.25 ml. The dose of MIF-I used in this experiment followed the practice of previous investigators [22]. The animals were injected in accordance with the following schedule: animals in the Day 1 duration group were injected for one day only, animals in the Day 3 group were injected each day for three days and in the Day 5 group animals were injected daily for five days.

The solutions were prepared each day just before injections. Synthetic MIF-I in 1 mg vials (Beckman lot no. 02708) had full potency at the time of testing. The vials were stored in dessicators at 5°C.

Behavioral procedure. Each rat was handled for 3 min each day for 7 days preceding the start of the experiment. Each rat was placed on the platform in the test box 90 min after injection following the practice of previous investigators [14,22]. The rats remained in the test box for 60 sec which is an optimal duration for eliciting a defecation response from albino rats [10]. The weight of the rat on the platform started an electronic clock and when the rat stepped down the latency was printed out. Only the first step-down latency was recorded as a measure of novelty-induced activity. When the rat had completed 60 sec in the test box it was taken out and the fecal boluses were collected and counted. After each trial the platform and grid floor were cleaned with distilled water and dried before the next rat was tested. The paper towel on the metal tray underneath the grid floor was also changed before each trial.

Animals in the Day 1 duration group were exposed to the test box for one day only: animals in Day 3 group were exposed each day for three days and animals in Day 5 group were exposed each day for five days.

Physiological procedure. Animals in each treatment duration group were sacrificed 91 min after injection on the final day scheduled for that duration group. When the rat had completed its last 60 sec in the test box it was removed and decapitated. The trunk blood was collected in heparinized tubes and centrifuged at 4000 rpm for 15 min. The plasma was collected in acid washed pre-marked vials, frozen and assayed for 11-OHCS the following day. Immediately after the blood was collected the brain of each rat was removed and weighed. The brain was sliced with a surgical blade on parafilm and was homogenized with a solid plastic trephine in a homogenizing tube. Each brain was homogenized in 5 ml of a freshly prepared solution of 3.7% perchloric acid and 0.1% sodium metabisulphate in double-distilled water. After the brain was homogenized it was kept frozen until all the samples were ready for centrifuging. The brain samples were then centrifuged at 4000 rpm for 15 min. The supernants were immediately collected in acid-washed vials and frozen and the deposits were again homogenized in another 5 ml of the solution. The homogenized samples were then cen-

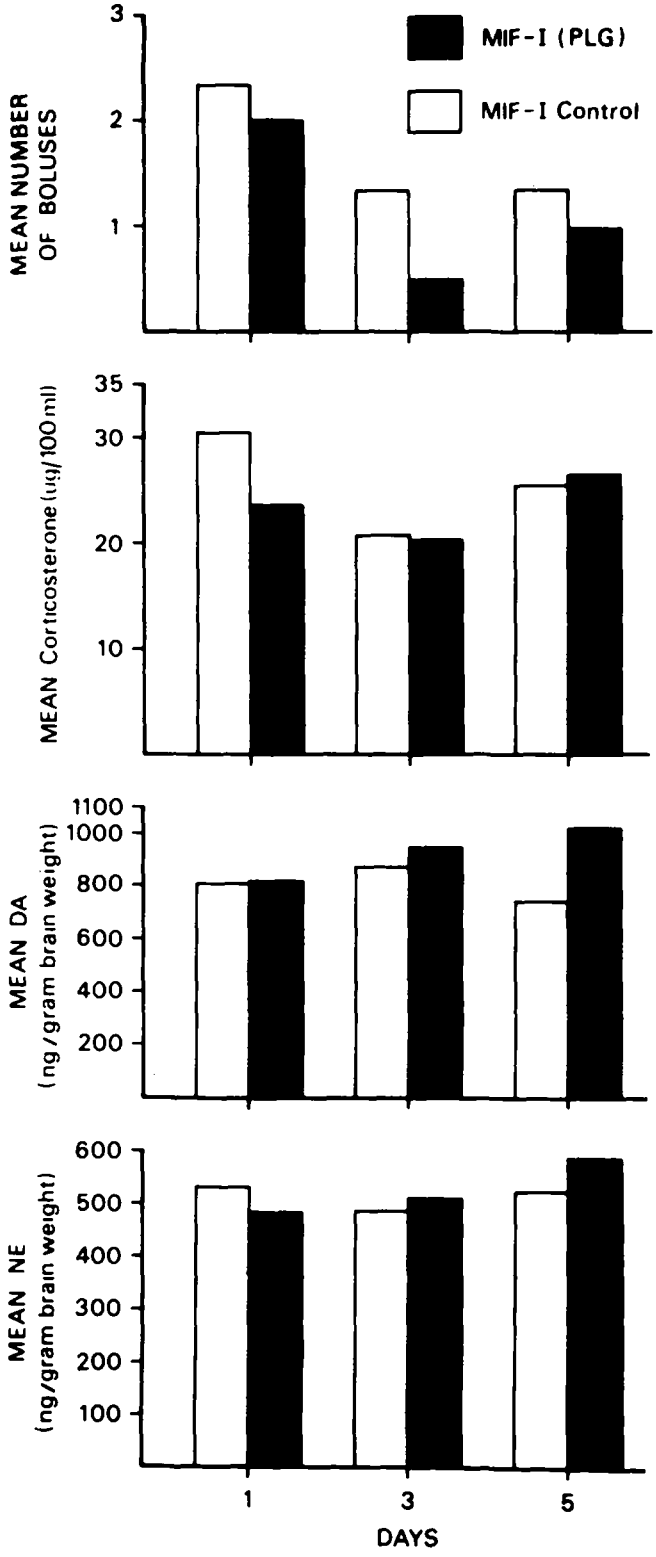

FIG. 1. Effects of MIF-I on novelty-induced defecation, plasma 11-OHCS levels (μg/100 ml), whole brain DA concentration (ng/gr wet brain tissue) and whole brain NE concentration (ng/gr wet brain tissue) on Days 1, 3 and 5 of novelty exposure.

trifuged for 15 min at 4000 rpm and the supernants were collected and frozen. The plasma and the brain samples were assayed within two days after collection.

Biochemical procedure. The plasma samples were assayed for 11-OHCS levels using the fluorometric method developed by Mattingly [31]. In the assay for plasma 11-OHCS at least two estimations were made on each sample and the mean was taken. The assay for brain DA and brain NE followed a fluorescence technique described by Haggendal [16] and Hinterberger [17]. The brain supernants were absorbed in alumina at pH 8.5 and diluted with IN sulphuric acid. As soon as the acid absorption was complete the samples were oxidised following the procedure described by Hinterberger [17]. Then the samples were examined for DA and NE at 330 and 400 mμ for excitation and 520 and 374 mμ for spectrum emission respectively. The assays for plasma 11-OHCS and brain CAs were carried out using a fluorescence spectrophotometer (Perkin-Elmer, Model MPF-4).

Statistical procedure. In order to satisfy the homoscedasticity assumption of the analysis of variance, the bolus frequencies and the stepdown latencies were subjected to a square-root ($X=\sqrt{X+1/2}$) transformation and a log ($\log_{10}$) transformation, respectively. Both measures were then analysed by a two-way analysis of variance (ANOVA) for repeated measures followed by multiple Scheffé comparisons [42].

RESULTS AND DISCUSSION

Results from the ANOVA indicated that MIF-I treatment relative to its control injection did not significantly affect either of the behavioral measures (latency and defecation) in animals exposed to the novel environment. Both treatment groups exhibited significant habituation of defecation responses, $F(2,20)=5.1$, $p<0.05$, over test days. The interaction effect of Treatments×Days was found to be significant for both DA, $F(2,20)=4.4$, $p<0.05$, and for NE, $F(2,20)=6.4$, $p<0.05$. Further analysis with ANOVA showed that the combined MIF-I with Novelty treatment significantly elevated both brain DA, $F(2,10)=5.7$, $p<0.05$, and brain NE, $F(2,10)=13.3$, $p<0.005$, concentrations over days. Individual Day comparisons showed that MIF-I treatment relative to its control injection significantly elevated on Day 5 concentrations of brain DA, $F(1,10)=19.7$, $p<0.005$, and brain NE, $F(1,10)=12.2$, $p<0.01$. These results support partially the findings of those investigators who observed an increase of brain DA after MIF-I treatment (Friedman *et al.*, 1973: Plotnikoff *et al.*, 1976) and adds further evidence that MIF-I treatment over days leads to a significant increase in whole brain NE concentrations. Statistical comparison between MIF-I and its control injection showed that the effect of MIF-I on plasma 11-OHCS approached significance on Day 1, $F(1,10)=3.9$, $p<0.07$. In Fig. 1 are shown the mean number of boluses, mean plasma 11-OHCS levels, mean whole brain DA and mean whole brain NE concentrations for MIF-I treated and the control-injected rats over days. The mean stepdown latency values with Standard Errors for stepdown latencies of MIF-I treated and MIF-I control treated rats were on Day 1=4.54 ± 0.69 and 6.42 ± 1.39, on Day 2=8.14 ± 2.07 and 5.70 ± 1.54, and on Day 3=4.51 ± 1.06 and 5.74 ± 1.56, respectively. Since stepdown latency scores were not significantly affected by either MIF-I or the novelty exposure treatment they have been omitted from Fig. 1.

Results of the Scheffé comparisons indicated that MIF-I treatment significantly elevated brain DA on Day 5 as compared with Day 1, $p<0.05$.

In general results suggest that MIF-I acts on both the dopaminergic and the noradrenergic nerve terminals and raises the whole brain content of DA and NE on successive days of the combined MIF-I with novelty treatment. The rise in whole brain DA and NE in MIF-I treated rats was accompanied by habituation of novelty-induced defecation responses but not of the 11-OHCS response. The control-injected rats, on the other hand, also showed a significant habituation of the 11-OHCS response over days, $F(2,10)=5.8$, $p<0.05$, but their brain DA and NE remained unchanged. Hence, it can not be claimed that this habituation of the 11-OHCS for the control-injected rats was mediated by the CAs which remained unchanged (Fig. 1).

Habituation of the defecation response over days and the inhibition of plasma 11-OHCS on Day 1 for the MIF-I treated rats is of interest. The MIF-I treated rats showed a tendency to decrease novelty-induced defecation on Day 2 compared with the defecation on Day 1. Also on Day 2 there was a slight tendency of brain DA and NE to rise (Fig. 1) although it can not be substantiated that the habituation in defecation of MIF-I treated rats was mediated by a concomitant rise in brain CAs over days of MIF-I treatment. Further, even though there was a reduction of plasma 11-OHCS levels in MIF-I treated rats relative to that of control injected rats, there was no concomitant rise in brain CAs on Day 1. Hence the possibility arises that MIF-I inhibited the plasma 11-OHCS response on Day 1 by itself acting on the adrenal cortex or by inhibiting pituitary MSH which has been demonstrated to possess significant steroidogenic activity [11,29]. The effect of MIF-I on elevated brain DA and NE might also be mediated by its inhibitory effect on MSH secretion [14,41]. This has been further substantiated by the recent demonstration that α-MSH treatment significantly decreased brain CA concentrations and sustained defecation responses in rats exposed to a novel environment [11].

EXPERIMENT 2

It has been reported [10] that Melatonin treatment, relative to its control-injection, facilitates the extinction of a passive avoidance response (PAR) and inhibits the defecation response elicited in the PA box during learning of the PAR. But the PA task involves both the shock and novelty components each of which is capable of inducing an 11-OHCS response in rats [7]. It therefore seems important to deconfound the novelty component from the shock component and to investigate the effect of Melatonin on novelty-induced defecation alone.

It was found in Experiment 1 that MIF-I significantly elevated whole brain DA and whole brain NE after 5 days of treatment and that this elevation in brain CAs was accompanied by a concomitant habituation of defecation. It has been reported by Spirtes, Kostrzewa and Kastin [39] that MIF-I decreases the synthesis of brain 5-HT, whereas Melatonin has been shown to increase significantly brain 5-HT, brain gamma aminobutyric acid (GABA) and brain pyridoxal kinase, an enzyme which is necessary for the biosynthesis of CAs, within half an hour of an IP injection [1]. It is therefore important to investigate the implication of the brain CAs (DA and NE) in the defecation response due to the combined Melatonin with novelty treatment. It has also been suggested by Motta and co-workers [32] that Melatonin may affect the stores and turnover of brain CAs because Melatonin loses its proven inhibitory action on histamine-induced stress in reserpine treated rats whereas DA sustains the inhibition of histamine-induced stress in reserpine treated rats [32]. Hence it seems probable that Melatonin leads to an increase in the turnover of whole brain DA and eventually inhibits stress responses.

Melatonin may also inhibit the pituitary adrenocorticotrophic hormone (ACTH) secretion by raising brain 5-HT [3,32] and thus may decrease the plasma 11-OHCS response to a stressor. Motta *et al.* [32] reported a significant reduction of plasma 11-OHCS after intraventricular administration of Melatonin whereas Barchas and co-workers [6] reported that subcutaneous administration of Melatonin does not modify either 11-OHCS or ACTH levels. In this experiment therefore the effect was investigated of Melatonin given IP on plasma 11-OHCS in rats exposed to a novel environment and its consequent effect on novelty-induced defecation responses over successive alternative days of exposure to a novel environment.

METHOD

Animals

Thirty-six naive male Wistar rats, 90–100 days old at the time of testing, were used. Animals were randomly assigned to three treatment duration groups: Day 1, Day 3 and Day 5, with 12 rats in each group. Six rats in each treatment duration group received the Melatonin treatment and the other six received a control injection. As in Experiment 1 animals were adapted to a 12:12 hr light:dark cycle for 4 weeks prior to the commencement of the experiment. The animals had ad lib access to food and water.

Apparatus

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

Procedure

Each rat was handled for 3 min each day for the 7 days prior to the beginning of the experiment.

Injection procedure. Rats in each daily sample received either an IP injection of Melatonin (250 μg/rat in a 0.25 ml solution) of 0.9% NaCl+0.01 M acetic acid+2% ethanol or an IP injection of 0.25 ml of this control solution without Melatonin. Animals in the Day 1 duration group were injected for one day only, animals in the Day 3 group were injected each day for three days and animals in the Day 5 group were injected daily for five days. Melatonin and its control solution were prepared each day before the injection procedure. Synthetic Melatonin (Grade A, Calbiochem) was kept in vials in dessicators at 4°C. The dosage of Melatonin used in this experiment was similar to the dosage used by previous investigators [10, 22, 30].

Behavioral procedure. Rats were placed in the test box 60 min after injection. This timing followed the practice of previous investigators [10,30]. The behavioral procedure for recording stepdown latencies and bolus frequencies was the same as was followed in Experiment 1. Only the stepdown latencies and bolus frequencies on the final test day for each group were used for statistical analysis.

Physiological procedure. Rats in each daily group were decapitated 61 min after injection on the final injection day for that group. When the rat had completed 60 sec in the test

box, it was immediately taken out, decapitated and the trunk blood collected for centrifuging at 4000 rpm for 15 min. The brains were removed and homogenised following the procedure described in Experiment 1.

Biochemical procedure. The procedures for collecting and assaying the blood plasma samples [31] and supernants of homogenised brain samples [17] were also the same as those followed in Experiment 1.

Statistical procedure. The stepdown latencies and the bolus frequencies were transformed ($\log_{10}$ and $X=\sqrt{X+1/2}$, respectively) in order to satisfy the homogeneity assumption of the ANOVA. As for Experiment 1, data analysis in this experiment was carried out using a two-way ANOVA for repeated measures followed by multiple Scheffé comparisons [42].

RESULTS AND DISCUSSION

Results from the ANOVA for repeated measures indicated that IP injection of Melatonin, relative to its control injection, significantly inhibited defecation responses over days, $F(1,10)=15.7$, $p<0.005$. The interactive effect of Melatonin and Trials on defecation was also significant, $F(2,20)=5.6$, $p<0.01$. Separate analysis of Melatonin treatment and the control-injection treatment showed that the defecation response of Melatonin treated rats did not habituate over days subsequent to Day 1 whereas the defecation of control-injected rats habituated significantly, $F(2,10)=9.9$, $p<0.005$, over days. Melatonin treatment compared to the control injection significantly inhibited novelty-induced defecation on Day 1, $F(1,10)=17.8$, $p<0.005$, and Day 3, $F(1,10)=25.2$, $p<0.005$, but not on Day 5 on which day defecation responses of control-injected rats had also habituated. Habituation of plasma 11-OHCS levels over days was found to be significant for control-injected rats, $F(2,10)=5.9$, $p<0.05$, and highly significant for Melatonin treated rats, $F(2,10)=21.4$, $p<0.0005$. Melatonin treatment, relative to its control-injection, reduced plasma 11-OHCS responses on Day 5, $F(1,10)=5.7$, $p<0.05$. Compared to the control-injection, Melatonin treatment significantly elevated whole brain DA, $F(1,10)=27.8$, $p<0.001$, over days. Results from single day comparisons by ANOVA showed that Melatonin treatment led to a significant rise in brain DA on Day 3, $F(1,10)=5.9$, $p<0.05$, and Day 5, $F(1,10)=8.1$, $p<0.01$. Whole brain NE, however, was not significantly changed by the combined Melatonin with novelty treatment over days even though there was a slight elevation after Melatonin alone. The mean number of boluses, mean plasma 11-OHCS levels, mean whole brain DA and mean whole brain NE concentrations for Melatonin treated and control-injected rats over Days 1, 3 and 5 are shown in Fig. 2.

The stepdown latency means and standard errors (sec) for Melatonin treated and control treated rats were: on Day 1=3.47 ± 0.53 and 4.52 ± 0.70, on Day 2=3.69 ± 0.41 and 9.53 ± 3.72, and on Day 3=8.98 ± 4.91 and 14.66 ± 7.99, respectively. As in Experiment 1 stepdown latencies did not add any information about Melatonin treatment or novelty exposure and they have not been included in Fig. 2.

Results of multiple Scheffé comparisons showed that Melatonin significantly reduced ($p<0.05$) plasma 11-OHCS on Day 5 compared with that on Day 1 (Fig. 2).

The above results suggest that novelty-induced defecation was inhibited by Melatonin treatment. Defecation of control-injected rats also habituated over days and on Day 5 Melatonin-treated and control-injected rats did not differ

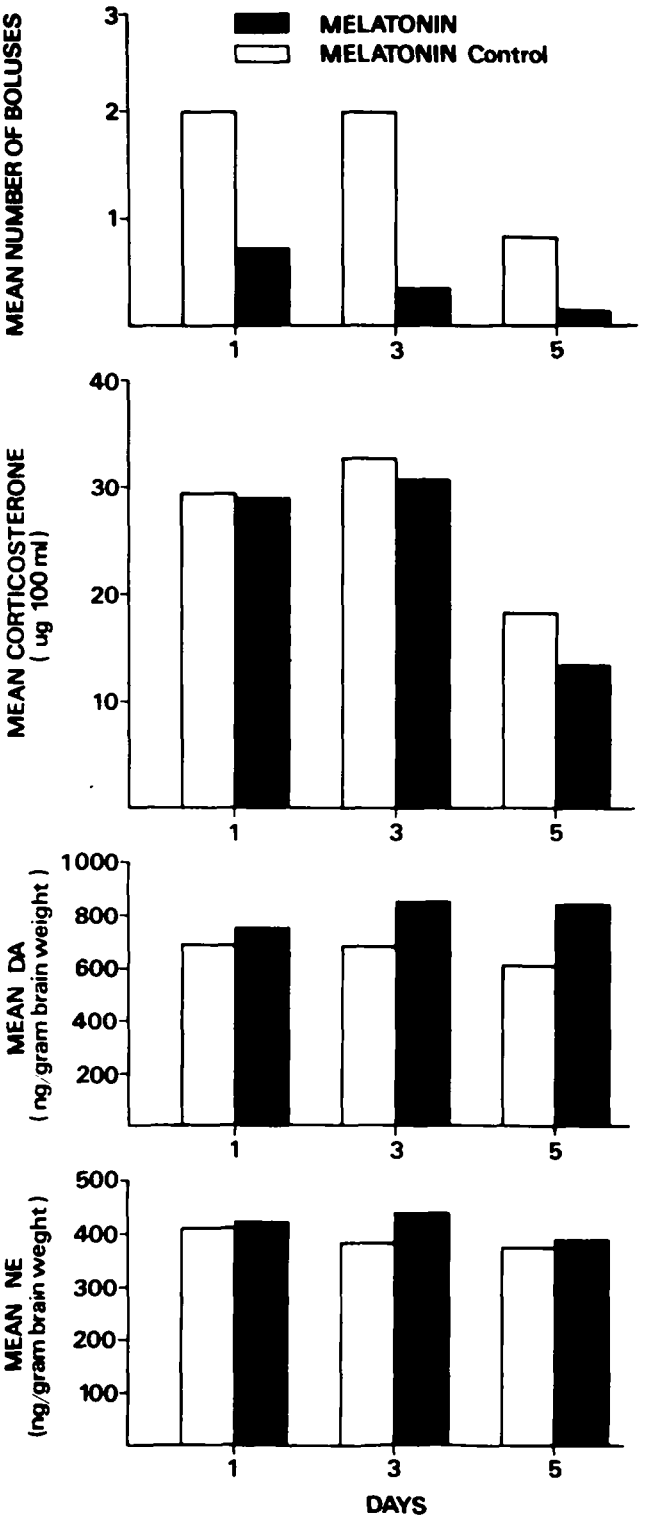

FIG. 2. Effects of melatonin no novelty-induced defecation, plasma 11-OHCS (μg/100 ml), whole brain DA concentration (ng/gr wet brain tissue) and whole brain NE concentration (ng/gr wet brain tissue) on Days 1, 3 and 5 of novelty exposure.

with respect to defecation responses. The significant inhibition of novelty-induced defecation was accompanied by a significant rise in whole brain DA. The significant habituation of plasma 11-OHCS levels and of defecation over days after Melatonin treatment verified an inhibitory effect of Melatonin on stress-induced responses in rats reported by several investigators [10,32]. The inhibitory effects of Melatonin on stress-induced responses seem to be mediated by an increased concentration of DA in whole brain after Melatonin treatment. These findings support the observations of Motta and associates [32] that intraventricular injections of both Melatonin and DA inhibit the histamine-induced plasma 11-OHCS response. They further observed that in animals pre-treated with reserpine, which significantly depletes brain CAs [9], Melatonin fails to inhibit the stress response induced by histamine. Hence it is most likely that in the present experiment Melatonin, by acting on the central dopaminergic nerve terminals, increases the turnover of DA in the whole brain and thus inhibits the novelty-induced defecation and habituates the novelty-induced plasma 11-OHCS response. Melatonin treatment, however, did not significantly elevate the NE concentrations in whole brain on successive days of treatment. Hence, it seems likely that the central dopaminergic system rather than the noradrenergic system is more involved in the Melatonin-induced inhibition of novelty-induced defecation and habituation of the novelty-induced plasma 11-OHCS response.

The present results suggest that IP Melatonin injection significantly inhibits novelty-induced defecation responses on successive days of treatment. The defecation response of control-injected rats habituates over days which is consistent with the finding of Datta and King [11] whereas the defecation of Melatonin treated rats relative to the controls seems to be significantly inhibited on Day 1 ($p<0.005$) and was consistently low on successive days. This indicates that Melatonin facilitates inhibition rather than habituation of novelty-induced defecation. This finding is similar to the observed inhibition of defecation responses in learning a PA task after Melatonin treatment [10]. In that study Melatonin not only reduced the novelty-induced defecation on Day 1 but also sustained the inhibited defecation over days. This sustained inhibition of novelty-induced defecation was exactly the opposite to the sustained disinhibition of novelty-induced defecation responses observed after α-MSH administration [11].

Consistent with its inhibitory effect on novelty-induced defecation responses Melatonin also significantly reduced the plasma 11-OHCS response over days. This significant habituation of plasma 11-OHCS by Melatonin on Day 5 supports the previous observations of the inhibitory effect of Melatonin on stress-induced defecation [10] and its inhibitory effect on stress-induced plasma 11-OHCS responses [32]. This suggests a tonic inhibitory effect of Melatonin on the pituitary-adrenal axis in stressful situations [32]. In the present experiment the inhibitory effect of Melatonin on defecation was significant on Day 1, whereas this was not the case for plasma 11-OHCS. Rather, there was a tendency towards increased 11-OHCS levels on Day 1 and Day 3 (Fig. 2). Novelty-induced plasma 11-OHCS was found to be habituated significantly by Melatonin on Day 5 compared to Day 1 ($p<0.05$).

Concomitant with its inhibitory effect on novelty-induced defecation and its habituatory effect on plasma 11-OHCS responses, Melatonin treatment also led to a significant elevation of whole brain DA over days relative to the control treatment (Fig. 2). Even though the increase in brain DA after Melatonin treatment was significant on Day 5 the DA level started increasing on Day 1 (Fig. 2). The significant rise in brain DA concentrations after Melatonin treatment over days relative to control treatment indicates that the inhibition of novelty-induced defecation and habituation of novelty-induced plasma 11-OHCS over days are probably mediated by the rise of DA over days of treatment with Melatonin. Motta and associates [32] have demonstrated that intraventricular injection of Melatonin and DA inhibit histamine-induced plasma 11-OHCS responses. In rats pretreated with reserpine which significantly depletes brain DA and NE [9], Melatonin failed to inhibit the histamine-induced stress response whereas DA retained the capacity to inhibit the stress response significantly. It is therefore most probable that Melatonin inhibits novelty-induced defecation and habituates novelty-induced 11-OHCS by raising the brain DA content over days of treatment, being significantly different on Day 5. In the present study brain NE was not found to be significantly raised by Melatonin although there was a slight rise in NE concentrations over days (Fig. 2).

The sustained novelty-induced defecation observed after α-MSH treatment [11] was found to be accompanied by a significant reduction of whole brain DA and NE concentrations and the combined MSH with novelty treatment was found to decrease significantly the content of brain NE over days. By contrast, the combined Melatonin with novelty treatment elevated brain DA. Hence it seems quite likely that whole brain NE concentration reduced by the combined MSH and novelty treatment is mainly responsible for the maintenance of novelty-induced defecation whereas whole brain DA concentrations, elevated by the combined treatment, mediate the sustained inhibition of novelty-induced defecation and habituation of the plasma 11-OHCS response. It has also been suggested by Motta *et al.* [32] that both Melatonin and DA should be considered as strong inhibitors of the pituitary-adrenal axis. They have further suggested that Melatonin may inhibit stress-induced responses by activating a central adrenergic pathway which inhibits ACTH secretion. It has also been demonstrated by Anton-Tay [1] that IP injection of Melatonin significantly increases brain 5-HT, GABA and pyridoxal kinase activity within 1 hr after injection. Hence the effects of Melatonin on the sustained inhibition of novelty-induced defecation and on habituation of plasma 11-OHCS over days may result from an elevation of brain DA or a combined elevation of brain 5-HT, GABA and brain DA.

It has been reported by Anton-Tay [1] that the rise of pyridoxal kinase activity may facilitate the biosynthesis of brain CAs since it is a very important enzyme in the process of GABA, 5-HT and CA synthesis. GABA itself is also considered by some as an inhibitory neurotransmitter [24,41]. Hence its involvement should also be considered in the inhibitory role played by Melatonin on stress-induced responses. The rise of brain DA after Melatonin may also be due to its possible direct action on central dopaminergic nerve terminals. (Increased 5-HT may also work at the nerve terminals and inhibit DA release.) Melatonin may also affect the central dopaminergic system directly or via its depletory effect on pituitary MSH.

Considering the points discussed above, it may be concluded that Melatonin (IP) leads to an inhibition of novelty-induced defecation and an habituation of plasma 11-OHCS responses probably by raising the concentrations of whole brain DA over successive days of the combined Melatonin

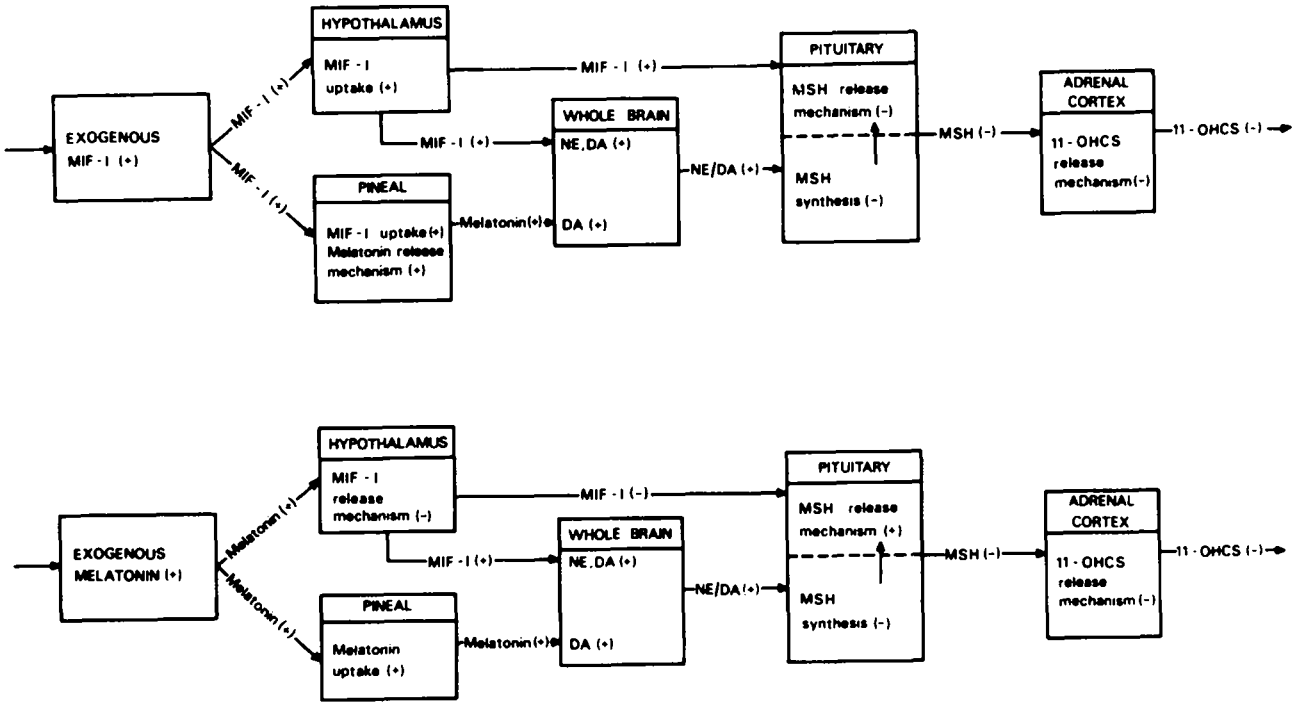

FIG. 3. A schematic diagram showing the effects of exogenous MIF-I and Melatonin on the release of 11-OHCS acting by way of the pineal, the hypothalamus and the pituitary and *via* concomitant changes in the concentrations of brain catecholamines.

with novelty treatment. The opposition between MSH and Melatonin on different behavioral paradigms may in part be mediated by a concomitant fall of the brain CAs after MSH [11] and by a concomitant rise of whole brain DA concentration after Melatonin treatment.

GENERAL DISCUSSION

From the two experiments it can be concluded that administration of MIF-I facilitates the habituation of novelty-induced defecation over days and that administration of Melatonin facilitates inhibition of novelty-induced defecation beginning on Day 1 exposure. Hence it seems that at the present dose level of 250 μg/rat/day of Melatonin is more potent than the same dose level of MIF-I in inhibiting defecation. Melatonin not only inhibited novelty-induced defecation on Day 1 but also sustained this inhibition on successive days of novelty exposure (Fig. 2). These findings also support the observations made by Datta and King [10] that Melatonin is capable of inhibiting defecation responses aroused during learning a PA task.

The present experiments also provide evidence that MIF-I mildly inhibits the plasma 11-OHCS response on Day 1 and that Melatonin contributes to the habituation of the plasma 11-OHCS response over successive days of treatment, particularly on Day 5. Treatment with MIF-I led to a mild inhibition of plasma 11-OHCS on Day 1 ($p<0.05$) whereas Melatonin slightly elevated 11-OHCS on Days 1 and 3 (Figs. 1 and 2). Administration of Melatonin was followed by a significant habituation of 11-OHCS relative to its control treatment on Day 5 whereas MIF-I did not result in a significant habituation effect on Day 5. These results suggest that Melatonin may be an inhibitor of the plasma 11-OHCS response to stressors which supports partially the previous findings of Motta *et al.* [32] that Melatonin, if injected intraventricularly, inhibits the histamine-induced 11-OHCS response in a single treatment dose. Motta *et al.* [32] also found that DA was equally effective in inhibiting the histamine-induced 11-OHCS response. In addition, they demonstrated that in rats pre-treated with reserpine, a depletor of monoamines, Melatonin treatment failed to inhibit the histamine-induced 11-OHCS response, whereas DA retained the capacity to inhibit the stress-response. Hence it has been suggested by Motta *et al.* [32] that probably brain DA, in addition to brain 5-HT which is significantly elevated by Melatonin treatment [1,2] may be involved in Melatonin-induced inhibition of stress responses. The present findings support this suggestion advanced by Motta *et al.* [32]. Of particular relevance are the present findings that Melatonin treatment leads to a significant elevation of brain DA over days of treatment and also a significant habituation of the plasma 11-OHCS response ($p<0.0005$). Melatonin treatment, relative to the control treatment, significantly elevated whole brain DA on Day 5. Hence the combined Melatonin with novelty effect on inhibition of defecation and on habituation of the plasma 11-OHCS response appeared to be concomitantly accompanied by, though not causally related to, a significant increase of whole brain DA over days. MIF-I treatment also led to a significant rise in brain DA relative to its control treatment. However, comparison across the two experiments suggests that the rise in brain DA after MIF-I was much more conspicuous than the rise of DA after Melatonin treatment (Figs. 1 and 2). Nevertheless the present findings indicate that Melatonin is more potent than MIF-I in exerting an inhibitory effect on novelty-induced

defecation and plasma 11-OHCS responses. These results suggest the possibility that in addition to the elevated level of brain DA, Melatonin by itself may be responsible for the maintenance of inhibited novelty-induced defecation and habituation of plasma 11-OHCS over days of treatment.

The observed elevation of brain DA after Melatonin and MIF-I is consistent with previous findings which have shown improvement of Parkinson tremor and subjective reports of well-being after Melatonin administration [3]. It is also consistent with the findings demonstrating improvement of Parkinson's tremor [4, 5, 23, 37] and of psychotic depression after MIF-I treatment [12]. In Experiment 1, MIF-I was also followed by a significant rise in brain NE concentrations over days: this was not found to be the case with Melatonin treatment in Experiment 2 even though Melatonin showed a slight tendency towards increased brain NE over days (Fig. 2). It is therefore more likely that the feelings of well-being and mood reported by depressed and normal persons after MIF-I and Melatonin treatment [3,12] are probably brought about by a significant rise in whole brain DA rather than NE or 5-HT. The rise in brain DA after Melatonin may be mediated by a rise in brain pyridoxal kinase activity which concomitantly follows Melatonin treatment [1] but the effect of MIF-I on brain GABA and pyridoxal kinase activity has yet to be established.

It has been reported by Datta and King [11] that treatment with α-MSH led to a significant reduction in whole brain DA and NE concentrations over days and that this reduction in brain CAs was accompanied by a sustained novelty-induced defecation response. Melatonin, which depletes pituitary MSH [41], and MIF-I, which inhibits pituitary MSH [18, 19, 23, 25], may therefore affect central CAs through an interactional process between MSH, MIF-I and Melatonin rather than by any one of these neuropeptides.

It has been claimed by several investigators [14, 24, 34, 36, 37] that MIF-I may have some catecholaminergic action in the CNS by itself. Hence in MIF-I treated animals exposed to a novel environment the release of brain CAs may be less than in control-injected animals exposed to the same environment. Working as a substitute inhibitory neuromodulator MIF-I therefore could lead to an increased turnover of brain DA and brain NE over days of treatment.

Melatonin has already been suggested as an inhibitory neuropeptide of pineal origin capable of inhibiting the pituitary-adrenal axis of the rat [32]. Hence the rise of brain DA after Melatonin may be due to the fact that Melatonin works in conjunction with DA at the nerve terminals in inhibiting the pituitary-adrenal response to stress [3, 27, 32]. It is possible that Melatonin increases brain DA and NE by inhibiting pituitary MSH. In either case there is low circulating MSH, a neuropeptide which has been found to decrease significantly brain concentrations of DA and NE and also to increase plasma 11-OHCS levels over days of treatment in the home cage condition [11]. Figure 3 summarizes such a system which is based on the observations of previous investigators [1, 3, 25, 27, 32, 40, 41] and the findings of the present experiments.

REFERENCES

1. Anton-Tay, F. Pineal-brain relationships. In: *The Pineal Gland*, edited by G. E. W. Wolstenholme and J. Knight. Edinburgh and London: Churchill Livingstone, 1971, pp. 213–220.
2. Anton-Tay, F., C. Chou, S. Anton and R. Wurtman. Brain serotonin concentrations: elevation following intraperitoneal administration of Melatonin. *Science* **162:** 277–278, 1968.
3. Anton-Tay, F., I. L. Diaz and F. Fernandez-Guardiola. On the effect of Melatonin upon human brain. Its possible therapeutic implications. *Life Sci.* **10:** 841–850, 1971.
4. Barbeau, A. and A. J. Kastin. Polypeptide therapy in Parkinson's Disease—a new approach. *5th International Symposia on Parkinson's Disease*, edited by W. Birkwayer. Vienna: Edition Roche, 1976.
5. Barbeau, A., M. Roy and A. J. Kastin. Double blind evaluation of oral l-prolyl-l-leucyl-glycine amide in Parkinson's disease. *Can. Med. Ass. J.* **114:** 120–122, 1976.
6. Barchas, F., F. DaCosta and S. Spector. Acute pharmacology of Melatonin. *Nature* **214:** 919–920, 1967.
7. Bassett, J. R., K. D. Cairncross and M. G. King. Parameters of novelty, shock predictability and response contingency in corticosterone release in the rat. *Physiol. Behav.* **10:** 901–907, 1973.
8. Bernard, B. K., J. R. Bercheck and D. A. Yutzey. Alterations in brain monoaminergic functioning associated with septal lesion induced hyperreactivity. *Pharmac. Biochem. Behav.* **3:** 121–126, 1975.
9. Carr, L. A. and K. E. Moore. Effects of reserpine and α-Methyltyrosine on brain catecholamines and the PA response to stress. *Neuroendocrinology* **3:** 285–302, 1968.
10. Datta, P. C. and M. G. King. Effects of α-MSH and Melatonin on passive avoidance and an emotional response. *Pharmac. Biochem. Behav.* **6:** 449–452, 1977.
11. Datta, P. C. and M. G. King. α-MSH and novelty-induced emotional responses: associated changes in plasma corticosterone and brain catecholamines. *J. comp. physiol. Psychol.*, submitted.
12. Ehrensing, R. H. and A. J. Kastin. Clinical investigations for emotional effects of neuropeptide hormones. *Pharmac. Biochem. Behav.* **5:** 89–93, 1976.
13. Fischer, P. A., E. Schneider, F. Jacobi and H. Maxion. Effect of melanocyte-stimulating hormone release inhibiting factor (MIF) in Parkinson's syndrome. *Eur. Neurol.* **12:** 360–368, 1975.
14. Friedman, E., J. Friedman and S. Gershon. Dopamine synthesis: stimulation by a hypothalamic factor. *Science* **182:** 831–832, 1973.
15. Ganong, W. F. *Review of Medical Physiology*, 7th edition. California: Lange, 1975, pp. 184–185.
16. Haggendal, J. An improved method of fluorometric determination of small amounts of adrenaline and noradrenaline in plasma and tissue. *Acta physiol. scand.* **59:** 246–254, 1963.
17. Hinterberger, H. The biochemistry of catecholamines in relation to Parkinson's disease. *Aust. N.Z. J. Med.* **1:** 14–18, 1974.
18. Kastin, A. J. and A. V. Schally. Autoregulation of release of melonocyte-stimulating hormone from the rat pituitary. *Nature* **213:** 1238–1240, 1967.
19. Kastin, A. J. and A. V. Schally. Control of MSH release in mammals. *Excerpta. Med.* **238:** 311–317, 1971.
20. Kastin, A. J., A. V. Schally, S. Viosca, L. Barrett and T. W. Redding. MSH activity in the pituitaries of rats exposed to constant illumination. *Neuroendocrinology* **2:** 257–262, 1967.
21. Kastin, A. J., A. V. Schally, S. Viosca and M. C. Miller. MSH activity in plasma and pituitaries of rats after various treatments. *Endocrinology* **84:** 20–27, 1969.
22. Kastin, A. J., M. C. Miller, L. Ferrell and A. V. Schally. General activity of intact and hypophysectomized rats after administration of Melanoctye-stimulating hormone (MSH), Melatonin and Pro-Leu-Gly-NH_2. *Physiol. Behav.* **10:** 399–401, 1973.
23. Kastin, A. J., N. J. Plotnikoff, A. V. Schally and C. A. Sandman. Endocrine and CNS effects of hypothalamic peptides and MSH. In: *Reviews in Neuroscience*, New York: Raven Press, 1976, pp. 111–148.

24. Kastin, A. J., N. P. Plotnikoff, C. A. Sandman, M. A. Spirtes, R. M. Kostrzewa, S. M. Paul, L. O. Stratton, L. H. Miller, F. Labrie, A. V. Schally and H. Goldman. The effects of MSH and MIF on the brain. In: *Anatomical Endocrinology*, edited by W. E. Stumpf and L. D. Grant, Basel: S. Karger AG, 1975, pp. 290–297.
25. Kastin, A. J., N. P. Plotnikoff, R. Hall and A. V. Schally. Hypothalamic hormones and the central nervous system. In: *Hypothalamic Hormones: Chemistry, Physiology, Pharmacology and Clincal Uses*. New York: Academic Press, 1975, pp. 261–268.
26. Kastin, A. J., S. Viosca and A. V. Schally. Regulation of melanocyte-stimulating hormone release. In: *Handbook of Physiology*, edited by E. Knobil and W. H. Sawyer, Vol. 4, 1974, pp. 551–562.
27. Kastin, A. J., S. Viosca, R. M. G. Nair, A. V. Schally and M. C. Miller. Interactions between pineal, hypothalamus and pituitary involving Melatonin, MSH-release inhibiting factor and MSH. *Endocrinology* **91**: 1323–1328, 1972.
28. Kastin, A. J., T. W. Redding and A. V. Schally. MSH activity in rat pituitary after pinealectomy. *Proc. Soc. exp. Biol. Med.* **124**: 1265–1277, 1967.
29. Lebovitz, H. E., S. Genuth and K. Pooler. Relationships between the structure and biological activities of corticotropin and related peptides. *Endocrinology* **79**: 635–642, 1966.
30. Martini, L. Behavioural aspects of pineal principles. In: *The Pineal Gland*, edited by G. E. W. Wolstenholme and J. Knight. Edinburgh and London: Churchill Livingstone, 1971, pp. 368–372.
31. Mattingly, D. A simple fluorometric method for estimation of 11-hydroxycorticoids in human plasma. *J. clin. Path.* **15**: 374–379, 1962.
32. Motta, M., O. Schiaffini, F. Piva and L. Martini. Pineal principles and the control of adrenocorticotropin secretion. In: *The Pineal Gland*, edited by G. E. W. Wolstenholme and J. Knight. Edinburgh and London: Churchill Livingstone, 1971, pp. 279–291.
33. Nair, R. M. G., A. J. Kastin and A. V. Schally. Isolation and structure of hypothalamic MSH release-inhibiting hormone. *Biochem. biophys. Res. Commun.* **43**: 1376–1381, 1971.
34. Plotnikoff, N. P. and A. J. Kastin. Oxotremorine antagonism by prolyl-glycine amide administered by different routes and with several anticholinergics. *Pharmac. Biochem. Behav.* **2**: 417–419, 1974.
35. Plotnikoff, N. P., A. J. Kastin, M. S. Anderson and A. V. Schally. DOPA potentiation by a hypothalamic factor, MSH release-inhibiting hormone (MIF). *Life Sci.* **10**: 1279–1283, 1971.
36. Plotnikoff, N. P., A. J. Kastin, M. S. Anderson and A. V. Schally. Deserpidine antagonism by a tipeptide L-propyl-L-leucyl-glycinamide. *Neuroendocrinology* **11**: 67–71, 1973.
37. Plotnikoff, N. P., F. M. Minard and A. J. Kastin. DOPA potentiation in ablated animals and brain levels of biogenic amines in intact animals after prolyl-leucylglycinamide. *Neuroendocrinology* **14**: 271–279, 1974.
38. Schildkraut, J. J. and S. S. Kety. Biogenic amines and emotion. *Science* **156**: 21–30, 1967.
39. Spirtes, M., R. Kostrzewa and A. J. Kastin. α-MSH and MIF-I effects on serotonin levels and accumulation in various rat brain areas. *Pharmac. Biochem. Behav.* **3**: 1011–1015, 1975.
40. Taleisnik, S. and M. E. Celis. Control of Melanocyte-stimulating hormone secretion. *Excerpta Med.* Amsterdam, 1973, pp. 100–106.
41. Taleisnik, S., M. E. Tomatis and M. E. Celis. Role of catecholamines on the control of melanocyte-stimulating hormone secretion in rats. *Neuroendocrinology* **10**: 235, 1972.
42. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill Inc., 1970.