

Increased δ Aminolevulinic Acid and Decreased Pineal Melatonin Production A Common Event in Acute Porphyria Studies in the Rat

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Abstract

Tryptophan (TRP) is the precursor of melatonin, the primary secretory product of the pineal gland. Hepatic heme deficiency decreases the activity of liver tryptophan pyrrolase, leading to increased plasma TRP and serotonin. As a paradox, patients with attacks of acute intermittent porphyria (AIP), exhibit low nocturnal plasma melatonin levels. This study using a rat experimental model was designed to produce a pattern of TRP and melatonin production similar to that in AIP patients.

Pineal melatonin production was measured in response to: (a) a heme synthesis inhibitor, succinylacetone, (b) a heme precursor, δ -aminolevulinic acid (Ala), (c) a structural analogue of Ala, γ -aminobutyric acid. Studies were performed in intact rats, perfused pineal glands, and pinealocyte cultures. Ala, succinylacetone, and γ -aminobutyric acid significantly decreased plasma melatonin levels independently of blood TRP concentration. In the pineal gland, the key enzyme activities of melatonin synthesis were unchanged for hydroxyindole-*O*-methyltransferase and decreased for *N*-acetyltransferase.

Our results strongly suggest that Ala overproduced by the liver acts by mimicking the effects of γ -aminobutyric acid on pineal melatonin production. These data could account for the low plasma melatonin in AIP. They also support the view that Ala acts as a toxic element in the pathophysiology of AIP. (*J. Clin. Invest.* 1996. 97:104–110.) Key words: porphyria • melatonin • δ -aminolevulinic acid • tryptophan • γ -aminobutyric acid

Introduction

Acute intermittent porphyria (AIP)¹ is a dominant inherited disease due to a defect in the third heme pathway enzyme porphobilinogen deaminase (E.C.4318, porphobilinogen [PBG] deaminase) (1). AIP is characterized by intermittent acute attacks of neurologic dysfunction that may affect the peripheral,

autonomic, or central nervous system. The pathophysiology is still poorly understood and biochemical findings are related to a lack of heme mainly in the liver. In plasma, during acute attacks of AIP, heme precursors, δ -aminolevulinic acid (Ala), and PBG, are increased (2).

Two major hypotheses, a lack of hemoproteins or a toxic effect of accumulation of heme precursors, have been suggested to explain neurologic disturbances, but few supporting results have been published.

Two recent studies have provided incremental advances: Firstly, in a chemically induced porphyric rat model, the plasma concentration and brain uptake of tryptophan (TRP) was enhanced and serotonin (5HT) synthesis increased in the nervous system (3, 4). These increases in TRP and 5HT concentrations were partly due to the hepatic heme deficiency decreasing the activity of the liver cytosolic enzyme (heme-dependent) tryptophan pyrrolase (5–7).

Secondly, a study on AIP in 12 women has demonstrated a rise in whole blood 5HT and total plasma tryptophan during the attacks whereas day and nighttime melatonin concentrations were dramatically decreased, although melatonin is produced from TRP (8). In this latter study, and as expected, injection of heme lowered heme precursors (Ala and PBG), TRP, and 5HT to normal levels (9) but did not increase melatonin concentrations (8).

The biochemical changes that cause the decrease in circulating melatonin remain unknown. Melatonin is synthesized in the pineal gland by the conversion of tryptophan to serotonin, which is then acetylated by *N*-acetyltransferase (NAT) to form *N*-acetylserotonin (10). *N*-acetylserotonin is subsequently converted to melatonin by the enzyme hydroxyindole-*O*-methyltransferase (HIOMT) (11). The pineal hormone is linked to the light–dark cycle through low daytime and high nighttime activity of NAT, the pineal rate-limiting enzyme (10). NE is the major neurotransmitter of the biochemical pathways controlling NAT activation via β 1 and α receptors on the pinealocyte (10). Melatonin is intimately related to the control of circadian rhythmic functions and their synchronization (12, 13). It has been implicated in human and mammalian physiology and pathology as well as in reproduction, chronobiological, and psychoneuroendocrine functions (13, 14). The physiological impact of melatonin secretion is in good correlation with clinical

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1. Abbreviations used in this paper: 5HT, serotonin; AIP, acute intermittent porphyria; Ala, δ -aminolevulinic acid; GABA, γ -aminobutyric acid; HIOMT, hydroxyindole-*O*-methyltransferase; NAT, *N*-acetyltransferase; PBG, porphobilinogen; SA, succinylacetone; TRP, tryptophan.

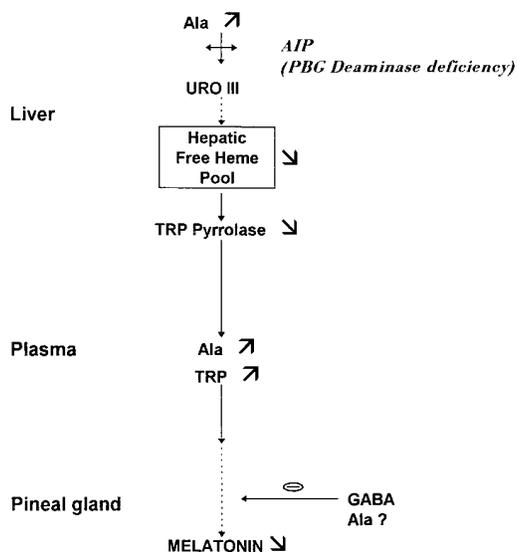


Figure 1. Paradox of low circulating melatonin and high TRP in acute attacks of AIP. A specific 50% PBG deaminase deficiency characterizes the AIP; it is shown as a horizontal arrow between Ala and uroporphyrinogen III (*URO III*). Bold arrows indicate increasing (↗) or decreasing (↘) amounts of metabolites occurring in acute attacks of AIP. Depletion of the hepatic free heme pool leads to a lack of tryptophan pyrrolase activity and high circulating TRP levels. However, there were low diurnal and nocturnal plasma melatonin concentrations that could be linked to a GABA-like effect of Ala.

cal features of acute attacks in AIP. Indeed, there is great intraindividual variability in the clinical expression of AIP, and the intermittent and cyclic nature of the attacks is well known. There is often a prodromal phase several days before the acute attack, with disturbances such as insomnia, depression, anorexia, and mood changes.

In this paper, we present a method suitable for analysis of the effect of heme deficiency and/or an excess of heme precursor Ala on melatonin production in rats. Ala is a structural analogue of the amino acid neurotransmitter γ -aminobutyric acid (GABA), therefore, we evaluated the effect of GABA under our experimental conditions (15).

This study was also designed to reproduce and try to explain in a rat model the coexistence of a low plasma melatonin concentration with a high circulating TRP level as previously observed in acute attacks of AIP (Fig. 1). We have studied the nocturnal response of rat pineal melatonin production to heme deficiency induced by a potent inhibitor, succinylacetone (SA), to Ala accumulation, and to their cumulative effect as it occurs in acute attacks of AIP. To do this, we measured the circulating concentrations of TRP and melatonin and the activities of the pineal enzymes NAT and HIOMT, *in vivo*, to investigate indirect effects (i.e., those mediated by the liver). We also performed *in vitro* investigations on perfused pineal glands and isolated pineal cell cultures to detect any direct effect of SA, Ala, and GABA on basal- and adrenergic-stimulated pineal melatonin production.

Methods

Chemicals. Ala, GABA, NE, and SA (4, 6-dioxoheptanoic acid) were purchased from Sigma Chemical Co. (St. Louis, MO). Melato-

nin was purchased from Fluka Chemie AG (Buchs, Switzerland), 125 I-labeled melatonin from Dositek (Orsay, France), rabbit antimelatonin antiserum and anti-Ig antiserum were from INRA (Tours, France).

Rat synchronization. 80 4–5-wk-old (100–120 g) male albino Wistar rats (IFFA-CREDO, L'Arbresle, France) were housed four rats per cage with food and water *ad lib*. 3 wk before each experiment, the rats were synchronized with a lighting regimen of alternating 12 h light and 12 h darkness. They were housed in a chronobiologic animal facility (Enceinte Autonome d'Animalerie, Ref E 110-SP-6, ESI Flufrance) with a light intensity of 3.1–3.8 μ W/cm² in the cages. The chronobiologic facility was equipped with equispaced, sound-proof, temperature-controlled ($21 \pm 1.0^\circ$ C) compartments provided with filtered air. Each compartment had its own lighting control. This procedure allowed us to explore several circadian stages at the same time (e.g., 7 and 19 h after light onset).

Experimental protocol. The rats were divided into five groups of 16 animals and synchronized for 3 wk. They were then fasted for 4 d with water *ad lib*. The control group was injected intraperitoneally with saline (1 ml/kg). The other four groups were given, respectively, SA (40 mg/kg), Ala (20 mg/kg), GABA (20 mg/kg), or SA + Ala (40 + 20 mg/kg). The amounts of chemicals injected were calculated from published data for Ala and GABA (15) and from data on the complete, stable inhibition of Ala-dehydrase activity with SA (16, 17). All the injections were given during each alternating light and dark phase, every 12 h. Half of the rats were killed after 1 d (two injections) and the others after 4 d (eight injections). Rats were killed by decapitation in the middle of the dark period (19 h after light onset) under a red light (0.4 μ W/cm² at the work surface) to avoid altering melatonin secretion. The blood was collected, centrifuged, and frozen at -70° C for melatonin and TRP measurements. The pineal glands were quickly removed and in each group two of them were frozen immediately and stored at -70° C for NAT and HIOMT activity assays. The remaining pineals were kept in oxygenated Krebs-Ringer solution in an iced water bath until their transfer to the perfusion chambers. The perfusion protocol has been described previously (18). Briefly, the perfusion system consisted of a plastic column closed with two pistons, a thermostatic bath which maintained the temperature at $37 \pm 0.5^\circ$ C, and a peristaltic pump. Pineal glands (two per chamber) were perfused for 3 h with Krebs-Ringer solution alone (pH 7.4, bubbled continuously with 95% O₂/5% CO₂) for the control group, and with Krebs-Ringer containing 1 mM Ala, 1 mM GABA, or 0.8 mM SA, adjusted to pH 7.4 for the other groups. Effluent perfusate fractions (10 min) were then collected into glass tubes over the next 5 h and stored at -20° C until assayed for melatonin. Lastly, the adrenergic response of the pineal glands was evaluated by perfusing them for 30 min (min 240–270) with 10^{-4} M NE in Krebs-Ringer (19).

Pinealocyte cell culture. Pineal cells were dispersed as previously described (20). Pineal glands from 3-wk-old rats were rapidly minced and rinsed in buffered saline: 0.1% BSA, 0.36 mM CaCl₂, 25 mM HEPES-NaOH, pH 7.3, 5 mM KCl, 137 mM NaCl, 0.7 mM Na₂HPO₄, penicillin (100 U/ml), and streptomycin (100 μ g/ml). Cells were dispersed by incubation with 6.4 U/ml trypsin and 8.1 U/ml DNase in saline solution for 30 min at 37° C. They were suspended in DME medium containing 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin. Aliquots (2×10^5 cells in 0.5 ml) were placed in sterile Eppendorf tubes. The culture was continued in an incubator at 37° C under an atmosphere of 5% CO₂ in air. All drugs (Ala, SA, and GABA) were dissolved in H₂O as 100 \times stock solutions. Experiments were performed on cells cultured for 24 h. Each drug was tested on cells cultured in medium alone and in medium containing 10^{-8} M isoproterenol (the β adrenergic stimulator). The cultured cells were pelleted (10,000 g for 10 s) and resuspended in 0.5 ml fresh medium. Drugs were added to final concentrations of 10^{-4} , 10^{-5} , or 10^{-6} M (5 μ l aliquots) and incubation continued for 6 h. The cells were removed by centrifugation (10,000 g for 10 s), and the medium was collected and frozen for melatonin assay.

Assays. Plasma Ala was measured by a fluorometric optimized

HPLC micromethod (21). Total plasma tryptophan was measured by HPLC and fluorometric detection (22). Pineal NAT and HIOMT activities were assayed according to Champney et al. (23). The intra-assay coefficients of variation were 11% for NAT and 14% for HIOMT ($n = 10$), and the interassay coefficients of variation were 13% for NAT and 16% ($n = 10$) for HIOMT. The melatonin concentrations in plasma and perfusate were measured directly by a modified RIA method, using ^{125}I -labeled melatonin (24, 25). The sensitivity of the assay was 5–10 pg/ml, the only significant cross-reactant was 6-hydroxymelatonin (0.1%). The intra-assay coefficient of variation was 8% ($n = 10$), and the interassay coefficient of variation was 11% ($n = 10$).

Statistics. All results are expressed as means \pm standard error. The effects of Ala, SA, and GABA on perfused pineals were measured as the melatonin production in a chamber expressed as a percentage of the mean concentration (“baseline level”) in the three fractions (210, 220, and 230 min) collected before addition of NE to the perfusion system. Students’ unpaired t test was used to analyze the experimental data.

Results

The plasma Ala concentrations and weights of rats during the 3 wk of the chronobiological experimental protocols are shown in Table I. The rats gained weight (+140%) during the 3 wk of synchronization and afterwards lost weight (–23%) during the 4-d fast. Plasma Ala concentrations were increased in the Ala, SA, and SA + Ala treated rats ($P < 0.001$).

Effect of Ala, SA, and GABA on circulating TRP and melatonin. Total plasma tryptophan was markedly decreased during activation of hepatic heme synthesis by Ala (–34%, $P < 0.01$), and increased during the inhibition of heme synthesis by SA (+24%, $P < 0.05$). Injections of GABA and SA + Ala did not modify the total plasma TRP (Fig. 2). Plasma melatonin concentrations were unaffected by treating the rats with Ala or SA for 1 d. However, 4-d-fasted rats had significantly lower nocturnal plasma melatonin levels when given Ala (–28%, $P < 0.01$), SA (–30%, $P < 0.01$), and GABA (–39%, $P < 0.01$). Ala + SA treatment was even more effective (–51%, $P < 0.005$) (Fig. 3).

In vivo effects of Ala, SA, and GABA on pineal HIOMT and NAT activities. The nighttime activity of pineal HIOMT was not altered by the drugs (Fig. 4 A). Nighttime pineal NAT activity was significantly decreased by all four treatments: Ala

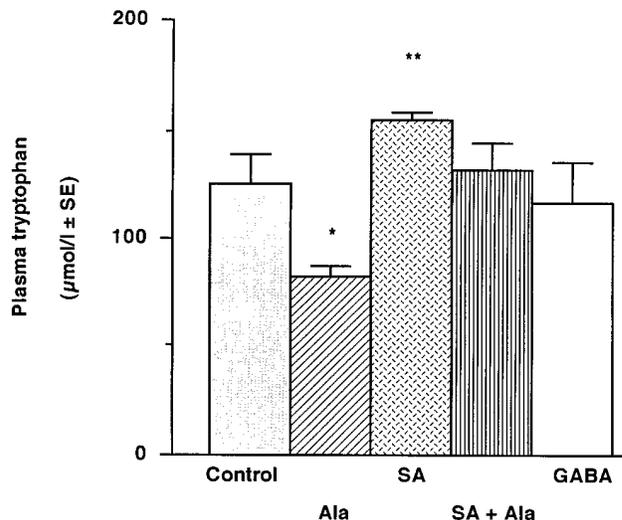


Figure 2. Plasma tryptophan concentrations. Effects of the heme precursor Ala, the heme inhibitor SA, a combination of Ala + SA, and GABA on total plasma tryptophan in rats fasted and given drug injections for 4 d ($n = 16$ per group). Ala (20 mg/kg), SA (40 mg/kg), and GABA (20 mg/kg) were injected intraperitoneally. Controls were injected with saline. Injections were given every 12 h during each alternating light and dark phase. Vertical bars show SEM. Significant difference from the saline controls: * $P < 0.01$; ** $P < 0.05$.

(–46%, $P < 0.005$), SA (–36%, $P < 0.01$), GABA (–39%, $P < 0.01$), and SA + Ala (–49%, $P < 0.005$) (Fig. 4 B).

Effect of in vitro perfusion of Ala, SA, and GABA on pineal HIOMT and NAT activities. The activity of pineal HIOMT was not altered by 4 h of drug perfusion (Fig. 5 A). The pineal NAT activity was significantly decreased after 4 h of perfusion with Ala (–30%, $P < 0.01$) or GABA (–38%, $P < 0.01$) but was unaffected by SA (Fig. 5 B).

Direct effects of Ala, SA, and GABA on melatonin production in rat perfused pineal glands. The rates of melatonin release from perfused pineal glands were measured for the last 5 h of an 8-h perfusion. Melatonin release decreased during the first 3 h of perfusion and reached fairly constant levels during the following 5 h (18). The mean concentration of melatonin between 200 and 230 min of perfusion was taken as the

Table I. Ala Concentrations and Weights of Rats during the Chronobiologic Experimental Protocol

	Day 0		Day 21		Day 25	
	Weight	Plasma Ala	Weight	Plasma Ala	Weight	Plasma Ala
	grams	µmol/liter	grams	µmol/liter	grams	µmol/liter
Control	112 \pm 4	0.010 \pm 0.01	252 \pm 6	0.013 \pm 0.01	198 \pm 12	0.018 \pm 0.01*
Ala	115 \pm 3	0.016 \pm 0.01	257 \pm 8	0.02 \pm 0.01	199 \pm 9	1.21 \pm 0.08*
SA	111 \pm 6	0.013 \pm 0.01	249 \pm 10	0.012 \pm 0.01	192 \pm 12	1.42 \pm 0.09*
SA 1 Ala	110 \pm 3	0.012 \pm 0.01	250 \pm 5	0.02 \pm 0.01	190 \pm 8	2.43 \pm 0.11*
GABA	108 \pm 4	< 0.009	247 \pm 6	0.010 \pm 0.01	186 \pm 7	0.02 \pm 0.01

Day 0: Before synchronization; Day 21: Synchronized rats before fasting and drug injection; Day 25: After 4 d of fasting and drug injection. $n = 16$ per group. Rats were kept in a chronobiologic animal facility with a light intensity of 3.1–3.8 $\mu\text{W}/\text{cm}^2$, compartments were equispaced, sound-proof, temperature-controlled (21 \pm 1.0°C), and provided with filtered air. Ala (20 mg/kg), SA (40 mg/kg), and GABA (20 mg/kg) were injected intraperitoneally. Controls were injected with saline. Results are mean \pm SEM. *Difference from pretreatment ($P < 0.001$).

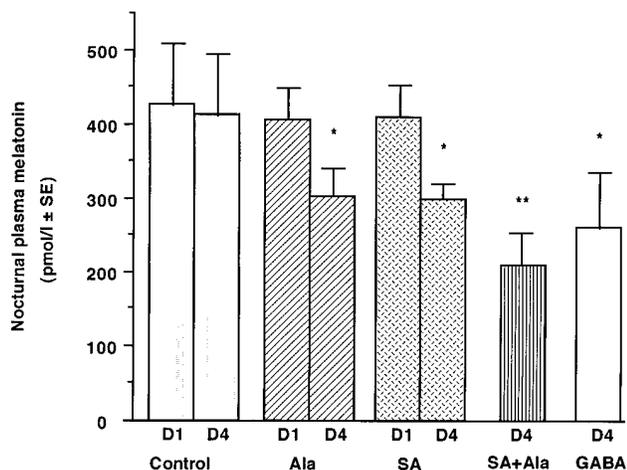


Figure 3. Nocturnal plasma melatonin concentrations. Effects of Ala, SA, Ala + SA, and GABA on nocturnal plasma melatonin concentrations in rats fasted for one (D1) and four (D4) days. Significant difference from the saline controls: * $P < 0.01$; ** $P < 0.005$.

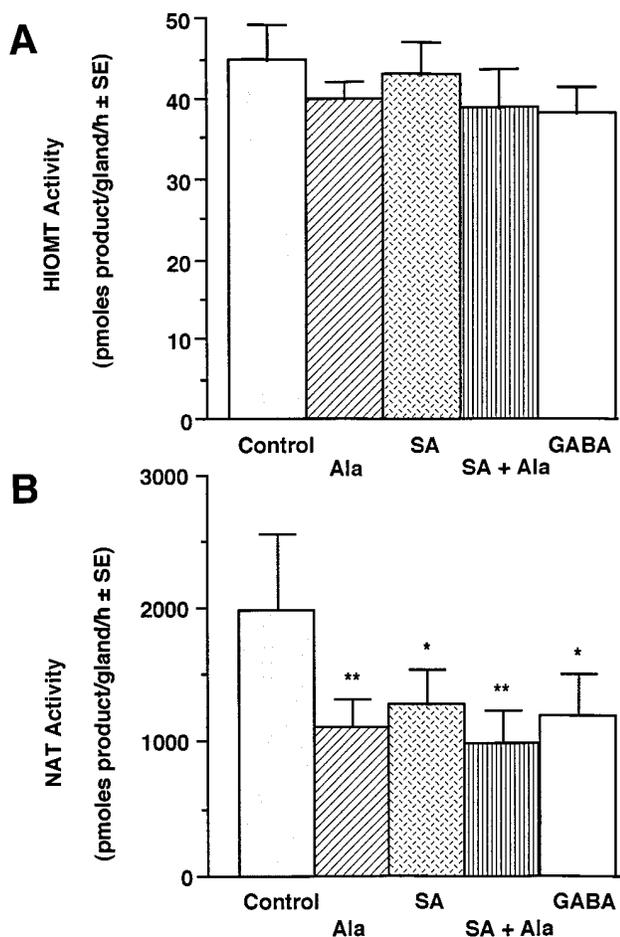


Figure 4. HIOMT and NAT activities after in vivo injection of Ala, SA, and GABA. (A) Effects of Ala, SA, Ala + SA, and GABA on the pineal HIOMT activity during the dark phase in rats fasted for 4 d. Results were not significantly different from saline controls. (B) Effects of Ala, SA, Ala + SA, and GABA on the pineal NAT activity during the dark phase in rats fasted for 4 d. Significant difference from the saline controls: * $P < 0.01$; ** $P < 0.005$.

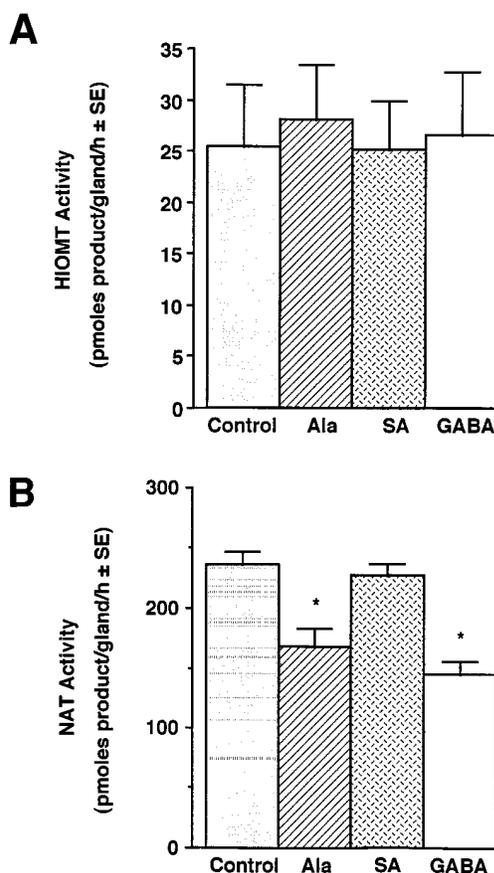


Figure 5. HIOMT and NAT activities after in vitro perfusion of pineal glands with Ala, SA, or GABA. (A) Effects of Ala, SA, and GABA perfused for 4 h on rat pineal HIOMT activity. Results were not significantly different from controls. (B) Effects of Ala, SA, and GABA perfused for 4 h on rat pineal NAT activity. Significant difference from controls: * $P < 0.01$.

baseline (Fig. 6). The responses of perfused isolated pineal glands to NE and drugs varied. SA did not significantly affect the amount, range, or the duration of the melatonin released by the pineal gland in response to NE ($305 \pm 18\%$ for 180 min) compared to controls ($322 \pm 22\%$ for 160 min). But both Ala and GABA canceled the NE stimulation, and, moreover, pineal gland response to NE was shorter in time and lower in amplitude: Ala, $180 \pm 8\%$ for 20 min; GABA, $160 \pm 12\%$ for 80 min; control, $322 \pm 22\%$ for 160 min.

Direct effects of Ala, SA, and GABA on melatonin production by rat pineal cell cultures. The melatonin content of pineal cell culture medium was unaffected by SA and significantly decreased by Ala and GABA compared to controls. Cells incubated with 10^{-8} M isoproterenol showed greater decreases in melatonin content (Table II).

Discussion

A previous study on patients suffering from acute attacks of AIP showed an increase in circulating TRP and a dramatic decrease in plasma melatonin, the pineal hormone synthesized from TRP (8). The inhibition of melatonin production observed in AIP could result either from a direct action of the

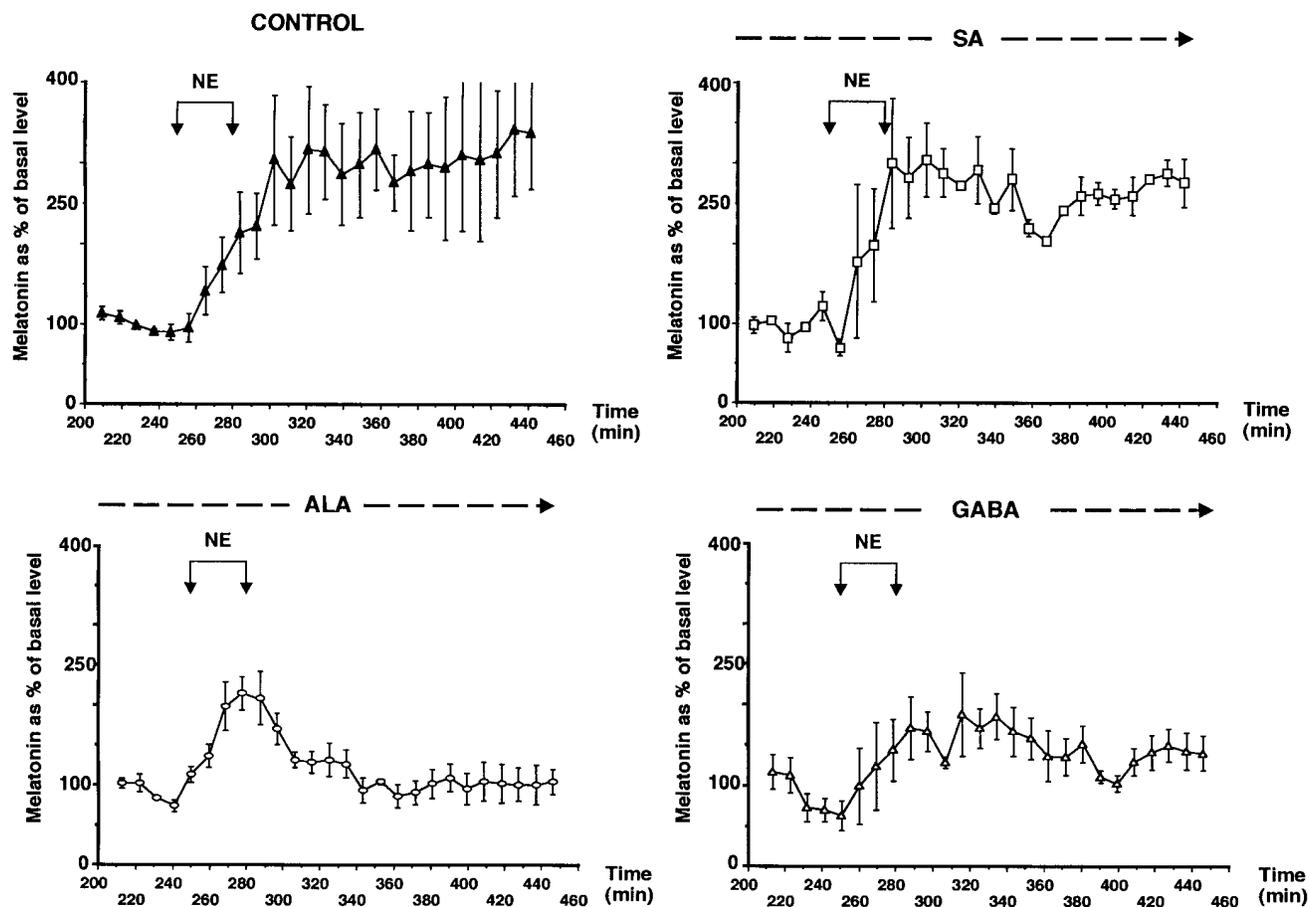


Figure 6. Patterns of melatonin release from perfused pineal glands. Effects of 0.8 mM SA, 1 mM ALA, and GABA on melatonin release from perfused pineal glands before, during, and after stimulation with 10^{-4} M NE. Each point is the mean \pm SE of data from three to six perfusion chambers (two pineal glands per chamber).

heme precursor Ala on pinealocytes, or it could result from a lack of hemoproteins either via a reduced transmission of light-dark signals from eyes to pineal receptors (hemoproteins are photoreactive) or via defects in hemoproteins such as cytochromes or NO synthase within the pineal gland. This reinforces a relationship between the clinical expression of this dominant genetic defect and environmental factors (1, 8). This study using a rat experimental model was designed to produce a pattern of TRP and melatonin production similar to that in AIP patients (8).

The decrease in total plasma TRP by Ala and its increase by SA are in agreement with the findings of Badawy et al. on the role of the hemoprotein TRP pyrrolase (5, 6). Giving rats the heme precursor Ala resulted in saturation of their liver TRP pyrrolase and activation of this enzyme; this enhanced TRP catabolism, reducing its circulating availability for brain uptake (7). As expected, the heme inhibitor, succinylacetone, had the reverse effect on circulating TRP (4). However, TRP production was not modified by the SA + Ala treatment, although the combination of both drugs produced a higher Ala

Table II. Melatonin Release from Pineal Cells in Culture

	Controls	Ala $10^{-4}M$	Ala $10^{-5}M$	Ala $10^{-6}M$	SA $10^{-4}M$	SA $10^{-5}M$	SA $10^{-6}M$	GABA $10^{-4}M$	GABA $10^{-5}M$	GABA $10^{-6}M$
Baseline melatonin production	3.93 \pm 0.25	2.36 \pm 0.16*	2.34 \pm 0.26*	3.18 \pm 0.31	3.95 \pm 0.31	3.84 \pm 0.29	4.01 \pm 0.19	2.18 \pm 0.21*	2.31 \pm 0.15*	2.97 \pm 0.34‡
Melatonin production after adrenergic stimulation	65.32 \pm 7.4	30.25 \pm 3.15*	34.12 \pm 5.21*	52.01 \pm 11.63	60.92 \pm 10.08	61.56 \pm 8.5	59.34 \pm 7.63	33.84 \pm 3.84*	31.78 \pm 2.32*	45.12 \pm 8.41‡

Ala, SA, and GABA were added at decreasing doses (10^{-4} , 10^{-5} , and $10^{-6}M$) before and after adrenergic stimulation with $10^{-8}M$ isoproterenol. Pineal cells were incubated without glucose for 6 h, then cells were removed by centrifugation (10,000g for 10 s), and melatonin was measured in the supernatant. All results are μ mol/liter \pm SE. *Difference from controls ($P < 0.005$); ‡difference from controls ($P < 0.01$).

accumulation than a single treatment: it could be hypothesized that exogenous Ala partially reversed SA inhibition on Ala dehydrogenase at a level that did not affect TRP production.

The low levels of melatonin found *in vivo* and *in vitro* were not correlated with the circulating tryptophan level but were clearly correlated with the high level of Ala. *In vivo* Ala and SA caused large decreases in both melatonin production and NAT activity. Ala was either exogenous (Ala treatment) or endogenous (inhibition of liver Ala-dehydrogenase activity by SA). Indeed, SA is the most potent inhibitor of Ala-dehydrogenase, the second enzyme of the heme pathway, which catalyzes the condensation of two molecules of Ala to form the monopyrrole, porphobilinogen (17).

The combination of both sources (Ala + SA) significantly enhanced the decrease in plasma melatonin in response to the higher concentration of circulating Ala. These data were confirmed by the internal checks on plasma Ala concentration, which showed increases with Ala, SA, and SA + Ala. A clear decrease in melatonin production *in vitro* was also induced by Ala, whereas SA did not alter either the concentrations of the hormone, the NAT activity, or the response to NE. Thus, the effect of SA *in vivo* and the lack of a direct effect on isolated pineal glands confirms that the action of SA is mediated by endogenous hepatic overproduction of Ala. Moreover, the pinealocyte cell culture experiments showed that endogenous Ala produced by the pineal itself after direct SA inhibition of heme synthesis had no effect on melatonin production, whereas exogenous Ala and GABA strongly decreased the production of melatonin.

For many years, there has been a suspicion that Ala is toxic, especially for nerve tissues, but this still remains to be demonstrated (26, 27). *In vivo*, Ala penetrates the blood-brain barrier poorly (2), and previous studies showed that Ala acted on isolated organs but not on whole animals (15, 28). This study is the first, to our knowledge, to demonstrate an effect of Ala *in vivo* and *in vitro* in rats. Pineal gland vascularization is outside that of the central nervous system and outside the blood-brain barrier. These results therefore support the hypothesis that Ala may have a toxic effect on melatonin production, and indicate that a lack of heme, hemoproteins, or porphyrins are not likely to be responsible for the decrease in pineal melatonin production.

Daya et al. (29) examined the effects of Ala on forebrain tryptophan and serotonin concentrations and on the pineal melatonin content. While Ala caused decreases in forebrain tryptophan and serotonin, it did not alter pineal melatonin levels. However, Ala was injected for only 1 d and food was provided *ad lib*. Our results also show no significant difference after treatment for 1 d. In a preliminary study (data not shown) Ala, SA, and GABA failed to exert measurable effects in fed animals. We began to see an effect on day 2, and the combination of fasting and treatment for 4 d unmasked the effect of Ala on the pineal gland.

The action of Ala is mainly due to its structural analogy with GABA (30). Ala can activate the GABA receptors on motor neurons and primary afferents (31). Studies on rabbit brain and rat jejunum have shown identical effects of Ala and GABA (15, 32).

While there has been little work on the relationship between Ala and melatonin synthesis and/or release, the effect of GABA on the pineal gland has been documented (33). There are great differences between birds and mammals (34). In

mammals, GABA has a global inhibitory effect on melatonin synthesis and its release (35). The addition of GABA to the medium inhibits melatonin release from rat pineal organotypic cultures (36). GABA inhibits NE-induced activation of NAT in ovine pineal glands (35). Thus, GABA behaves as a modulating inhibitory signal in rat, bovine, and ovine pineal glands and is released by exposure to the natural transmitter NE and impairs NE postsynaptic effects (36). The inhibition of noradrenergic activity by GABA has been shown to be post and presynaptic in rat pineals (36). Our perfusion system was able to preserve post and presynaptic domains since the nerve terminals innervating the pineal gland are known to need >24 h to degenerate (37).

Our results show similar nighttime decreases in melatonin release *in vivo* in rats given GABA or Ala. This decrease is associated with a decrease in pineal NAT activity but not in HIOMT activity. *In vitro* perfusion of pineal glands from GABA- and Ala-treated rats and pineal cell cultures showed impaired response of pineal melatonin production after NE stimulation. The adrenergic innervation of the pineal gland plays an important part in the circadian rhythm of melatonin via NAT activity. Therefore, our results strongly suggest a mimetic decreasing effect of Ala and GABA on pineal NAT activity mediated by impairment of the noradrenergic stimulation. These data could explain the low nocturnal plasma melatonin concentration in rats.

In conclusion, Ala, SA, and GABA cause decreased production of pineal melatonin in intact rats. The patterns of TRP and melatonin production are similar to those in AIP patients. We suggest that a decrease in melatonin production is induced by an increase in Ala concentration and not by a lack of hemoproteins. We therefore propose that the biochemical mechanism could be a "GABA-like effect" of Ala that decreases NAT activity and blocks the pineal response to β adrenergic stimulation. We also argue for the role in rats of Ala combined with fasting in metabolic disturbances not only in isolated organs but also in the intact animal. These findings could help to improve our knowledge of the clinical expression of acute intermittent porphyria in humans. Finally, the pathophysiological significance of these findings requires further examination to explore any relationship between this frequent autosomal dominant genetic disease and the influence of environmental disturbances on its clinical manifestations.

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