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Prolonged Hypothermia After Phenobarbital-Induced Anesthesia in Adrenalectomized Rats

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JANOCKO, L. AND M. J. MYCEK. *Prolonged hypothermia after phenobarbital-induced anesthesia in adrenalectomized rats.* PHARMACOL BIOCHEM BEHAV **24**(6) 1651–1658, 1986.—Adrenalectomized rats are hypersensitive to the hypothermic as well as hypnotic effects of phenobarbital. The exaggerated hypnotic response is more pronounced in the chronic (10 day) than the acute (1 day) adrenalectomized rat and is reversed by glucocorticoid replacement. The prolonged hypothermia to a hypnotic dose of phenobarbital occurs in adrenalectomized but not adrenodemedullated rats. This hypersensitivity is characterized by an impaired ability to regain normal body temperatures for as long as 24 hours after regaining the righting reflex. The prolonged hypothermia is prevented by prior treatment with glucocorticoids but not mineralocorticoids. There appears to be no gross alteration in disposition of phenobarbital following adrenalectomy suggesting that the prolonged duration of hypothermia is not a consequence of accumulation of the drug.

Phenobarbital Adrenalectomy Hypothermia Glucocorticoids Hypnotic response

ADRENALECTOMY is associated with a diminished ability to adapt to a variety of stressful stimuli including starvation and extremes in environmental temperature. It is well known that adrenalectomized animals are also highly susceptible to depressant agents such as the barbiturates [16, 30, 38, 39, 44]. The exaggerated response to some barbiturates, especially those whose termination of action depends on metabolic conversion, can be explained by altered disposition. Hepatic mixed function oxidase activity is low in adrenal insufficiency [15], consequently the biotransformation of pentobarbital and hexobarbital is reduced and accumulation of the drug is correlated with the prolonged hypnotic response [30,31].

A prolonged anesthetic response to barbital, which is metabolized slowly, if at all [25], is also observed following adrenalectomy. Komiya and Shibata [16] suggested that the hypersensitivity to barbital may result from a change in distribution kinetics. They reported that while the initial uptake of barbital was the same in the brains of adrenalectomized and sham operated mice, the drug persisted for longer periods of time in the brains of adrenalectomized animals. Their investigation did not rule out other factors such as alterations in central nervous system processes or potentiation of hypnotic effects due to hypothermia. The possibility that central processes may be involved in the hyperresponsiveness to phenobarbital was suggested by work in this laboratory. Chronic administration of phenobarbital (800 μg, 2 times daily for 5 days) intracerebroventricularly (ICV) into rats prepared with implanted cannulae, decreased the duration of the loss of the righting reflex, that is, tolerance developed [27]. Similarly, rats adrenalectomized only 1 day prior to ICV phenobarbital according to the same chronic regimen became tolerant. However, rats adrenalectomized 10–14 days prior to chronic phenobarbital administration became progressively more sensitive to the ICV barbiturate as demonstrated by a longer duration of the loss of righting reflex (Villano *et al.*, in preparation).

We were therefore interested in determining whether adrenalectomized rats receiving phenobarbital intraperitoneally (IP) would respond similarly. In addition to the duration of hypnosis, investigators have measured time to onset of loss of righting reflex [27], lethality and hypothermia [14] following barbiturate administration as criteria for assessing sensitivity to these drugs. We found that adrenalectomized rats responded to the IP administration of phenobarbital not only with a longer duration of loss of righting reflex, but also with a prolonged hypothermia. Both of these hypersensitivities were counteracted by the administration of adrenal steroids.

METHOD

Male Sprague-Dawley rats (125–150 g) were purchased from Taconic Farms, Germantown, NY. They were housed in the animal care facilities on a 12 hour light/dark cycle and fed standard rat chow ad lib. Body weights of sham operated

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TABLE 1

EFFECT OF DURATION OF ADRENALECTOMY AND ADRENODEMEDULLATION ON THE HYPNOTIC RESPONSE TO PHENOBARBITAL

	Onset (min)	Sleep Time (min)	n
A.			
Sham	13.3 ± 0.5	131.1 ± 4.1	73
Adrex-1 day	8.9 ± 0.5*	169.1 ± 7.7*	33
Adrex-10 day	9.4 ± 0.4*	207.4 ± 8.8*†	43
B.			
Sham	12.2 ± 0.9	162.1 ± 15.4	5
Adrenodemedullated-10 day	11.3 ± 2.0	143.9 ± 14.0	4

Animals were adrenalectomized (adrex) either 1 or 10 days prior to administration of phenobarbital (110 mg/kg, IP). Adrenodemedullation was performed 10 days prior to phenobarbital challenge. Values are expressed as means ± SEM.

*$p<0.05$ Compared to corresponding sham operated group.

†$p<0.05$ Compared to 1 day adrenalectomized group.

TABLE 2

EFFECT OF GLUCOCORTICOIDS ON THE HYPNOTIC RESPONSE TO PHENOBARBITAL

	Onset (min)	Sleep Time (min)
Sham		
Vehicle	12.2 ± 0.6	121.7 ± 14.3
Corticosterone	14.3 ± 1.5	121.2 ± 9.7
Dexamethasone	14.7 ± 1.3	95.5 ± 8.0
Adrex		
Vehicle	8.5 ± 0.8*	191.3 ± 16.1*
Corticosterone	10.5 ± 0.4	136.2 ± 12.7†
Dexamethasone	11.8 ± 0.6	108.8 ± 6.3†

Rats were adrenalectomized (adrex) or sham operated 10 days prior to administration of phenobarbital (110 mg/kg, IP). Dexamethasone or corticosterone were administered SC on alternate days for 10 days in doses of 0.1 mg and 0.5 mg, respectively. There were between 4 and 8 animals in each group. Values are expressed as means ± SEM.

*$p<0.05$ Compared to corresponding sham operated animals.

†$p<0.05$ Compared to corresponding adrenalectomized animals.

TABLE 3

EFFECT OF MINERALOCORTICOIDS ON THE HYPNOTIC RESPONSE TO PHENOBARBITAL

	Onset (min)	Sleep Time (min)
Sham		
Vehicle	10.6 ± 0.4	140.8 ± 10.3
Aldosterone	12.7 ± 0.7	153.6 ± 15.0
18-HDC	12.0 ± 0.2	134.3 ± 8.8
Adrex		
Vehicle	8.7 ± 0.6*	206.5 ± 21.7*
Aldosterone	8.4 ± 0.7*	183.1 ± 12.4
18-HDC	8.1 ± 0.4*	200.6 ± 23.8*

Rats were adrenalectomized (adrex) or sham operated 10 days prior to administration of phenobarbital (110 mg/kg, IP). Aldosterone or 18-hydroxydeoxycorticosterone (HDC) were administered SC on alternate days for 10 days in doses of 0.25 and 0.5 mg, respectively. There were between 7 and 9 animals in each group. Values are expressed as means ± SEM.

*$p<0.05$ Compared to corresponding sham operated animals.

animals at the end of a 10 day chronic experiment ranged from 175–240 g. Body weights of adrenalectomized animals in chronic experiments ranged from 165–240 g.

Surgical Procedures

Bilateral adrenalectomy was performed via the dorsal approach under ether anesthesia; sham operations consisted of manipulation of both adrenal glands. Adrenalectomized rats were given 0.9% saline to drink, whereas sham operated animals received tap water. Adrenalectomy was confirmed by measuring both serum corticosterone levels and thymus weights as well as by inspection at autopsy. Serum corticosterone was determined by radioimmunoassay [19] using antiserum obtained from Endocrine Sciences (Tarzana, CA). This antiserum is highly specific and shows minimal cross-reactivity with cortisol. The steroid was extracted from the serum prior to radioimmunoassay (Radioimmunoassay Procedure No. B21-42, Endocrine Sciences, Tarzana, CA). Corticosterone determinations were corrected for efficiency of extraction. In separate experiments begun at 9:00 a.m., sham operated animals had mean corticosterone levels of 3.2±0.5 μg/100 ml (mean±standard error, n=6) and 7.4±1.6 μg/100 ml (n=5). The variability between experiments depended on the handling of the animals as well as time of blood sampling but sham operated animals always had serum corticosterone levels significantly higher than those of adrenalectomized animals in which they were at the limit of sensitivity of the assay (<1.0 μg/100 ml). Adrenodemedullation was performed by gentle expulsion of adrenal medullary tissue through an incision made in the gland. Serum corticosterone levels were unaltered as a result of this surgical procedure.

Pharmacological Treatments

Phenobarbital sodium (Baker) was dissolved in distilled water (44 mg/ml) and injected intraperitoneally in a dose of 110 mg/kg. This dose was chosen because it consistently produced a loss of righting reflex. Corticosterone-21-acetate, dexamethasone, aldosterone and 18-hydroxydeoxycorticosterone (Sigma Chemical Co.) were dissolved in a few drops of acetone and mixed with corn oil before the acetone was evaporated with a stream of air. Rats were injected subcutaneously (SC) on alternate days with the appropriate steroid in a volume of 0.2 ml. The doses depended on the potency of the steroid and are described in the text. Control rats received an equal volume of vehicle.

Behavioral and Physiological Responses

The hypnotic response to the barbiturates was monitored by noting the times at which the animal lost and regained the righting reflex; this interval is sometimes referred to as

"sleep time." Anesthetized animals were periodically tested (approximately every 15 minutes) for restoration of the righting reflex, i.e., completion of three successive rightings in one minute (awakening). The interval between phenobarbital administration and loss of righting reflex (onset time) was also noted.

Rectal temperatures were measured at various time points corresponding to the hypnotic response to phenobarbital using a rectal thermistor probe (Telethermometer, Yellow Springs Instrument Co.).

Serum Collection and Phenobarbital Determinations

Rats were decapitated at various time points relative to the hypnotic response to phenobarbital. Trunk blood was collected into test tubes kept in an ice bath; serums were stored at −20°C for subsequent determinations of phenobarbital and corticosterone.

Serum phenobarbital concentrations were measured directly by radioimmunoassay using antiserum to phenobarbital provided by Dr. Edward J. Flynn, UMDNJ-New Jersey Medical School, Newark, NJ. The major metabolite of phenobarbital, p-hydroxyphenobarbital, does not cross react with this antiserum. [34].

Statistical Analyses

Student's t-test (two tailed) was used to compare the means of two experimental groups, with $p<0.05$ taken as the level of statistical significance. More than two experimental groups were compared by analyses of variance (one way). For comparison of individual group means, Duncan's range test with the modification of Kramer [18] for unequal replicates was employed.

RESULTS

Hypnotic Response to Phenobarbital

Adrenalectomy lengthened the duration of the hypnotic response to IP phenobarbital in accord with reports of other investigators. In order to differentiate the acute and chronic effects of adrenalectomy, rats were administered a hypnotic dose of phenobarbital, 110 mg/kg, at 1 or 10 days postsurgery. One day postsurgery was chosen to examine acute effects of adrenal insufficiency because by 24 hours endogenous circulating glucocorticoids have decreased to less than 10% of preoperative levels [4].

The degree of responsiveness to phenobarbital-induced hypnosis was influenced by the duration of the adrenalectomized state. Rats adrenalectomized 10 days prior to phenobarbital challenge were more sensitive to the hypnotic effects of the drug as indicated by the longer duration of loss of righting reflex (sleep time) compared to controls. The increased hypnotic response was not as pronounced in rats adrenalectomized for 1 day, however both groups of adrenalectomized animals had significantly shortened times to onset of anesthesia. Hypnotic response data are summarized in Table 1A. Because surgical adrenalectomy involves extirpation of the adrenal medulla and therefore decreases the stress-induced release of catecholamines, we considered the possibility that adrenomedullary catecholamines altered the sensitivity to phenobarbital's hypnotic effects. As shown in Table 1B, rats adrenodemedullated 10 days prior to phenobarbital challenge did not show any evidence of increased hypnotic sensitivity. These data suggested the involvement of the adrenal cortex in the alteration of sensitivity to phenobarbital. Several adrenal steroids possessing glucocorticoid or mineralocorticoid activity were therefore examined for their ability to prevent the development of sensitivity to phenobarbital following adrenalectomy.

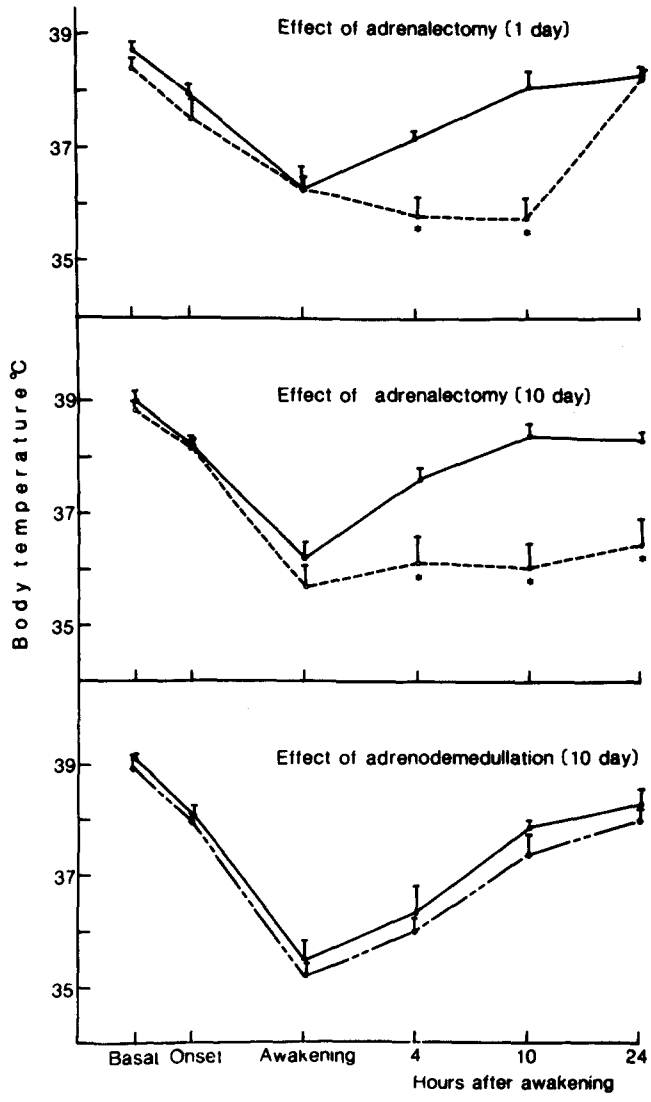

FIG. 1. (A, B, C) Hypothermic response to phenobarbital after adrenalectomy or adrenodemedullation. Rats were adrenalectomized, adrenodemedullated or sham operated prior to challenge with phenobarbital (110 mg/kg, IP). Rectal temperatures were measured at various time points corresponding to the hypnotic response to phenobarbital. There were between 4 and 8 animals in each experimental group. Values are expressed as means±SEM. Sham ●; Adrex ○; Adrenodemedullated ◆ *$p<0.05$ Compared to corresponding sham operated group.

Chronic replacement therapy with either corticosterone, the predominant glucocorticoid of the rat [5], or dexamethasone, a synthetic glucocorticoid lacking mineralocorticoid activity [35], proved effective in preventing the prolongation of narcosis induced by phenobarbital in 10 day adrenalectomized rats (Table 2). Though not statistically significant, there was also a tendency for glucocorticoid treatment to prolong the times to onset of the loss of righting

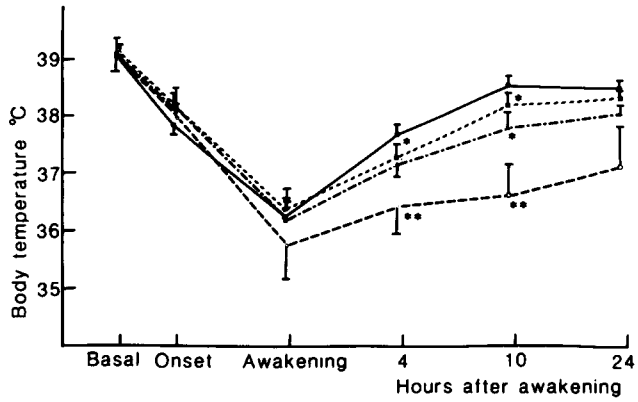

FIG. 2. Effect of glucocorticoids on the hypothermic response to phenobarbital. Rats were adrenalectomized or sham operated (●) 10 days prior to challenge with phenobarbital (110 mg/kg, IP). Adrenalectomized animals were administered either corticosterone (0.5 mg, SC, ▲), dexamethasone (0.1 mg, SC ■) or vehicle (○) every other day, beginning the day after surgery. Rectal temperatures were measured at various time points corresponding to the hypnotic response to phenobarbital. There were between 4 and 9 animals in each experimental group. Values are expressed as means±SEM. *$p<0.05$ Compared to the vehicle-treated adrenalectomized group. **$p<0.05$ Compared to the sham operated group.

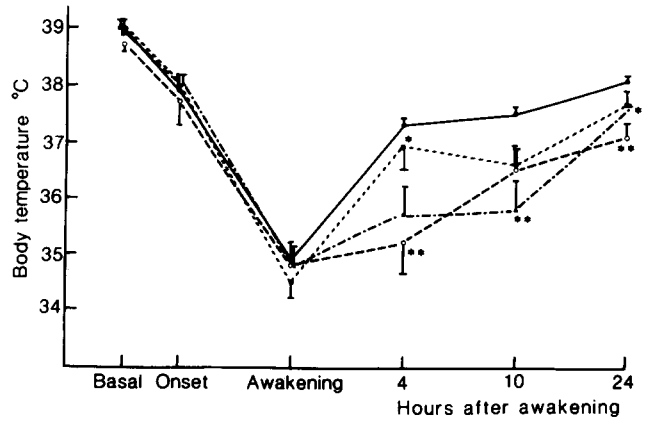

FIG. 3. Effect of mineralocorticoids on the hypothermic response to phenobarbital. Rats were adrenalectomized or sham operated (●) 10 days prior to challenge with phenobarbital (110 mg/kg, IP). Adrenalectomized animals were administered either aldosterone (0.25 mg, SC, ■), 18-hydroxydeoxycorticosterone (0.5 mg, SC, ▲) or vehicle (○) every other day, beginning the day after surgery. Rectal temperatures were measured at various time points corresponding to the hypnotic response to phenobarbital. There were between 3 and 9 animals in each experimental group. Values are expressed as means±SEM. *$p<0.05$ Compared to the vehicle-treated adrenalectomized group. **$p<0.05$ Compared to the sham operated group.

TABLE 4

EFFECT OF ADRENALECTOMY ON SERUM PHENOBARBITAL LEVELS (PB, μg/ml)

	Time after PB	PB Levels (μg/ml)	n
Sham (1 day)	132.3 ± 12.3 (min)	88.8 ± 13.6	5
Adrex (1 day)	174.1 ± 17.3 (min)*	82.0 ± 11.7	5
Sham (10 day)	127.7 ± 8.4 (min)	99.0 ± 7.6	9
Adrex (10 day)	190.7 ± 13.6 (min)*	104.2 ± 7.6	8
Sham (1 day)	48 hours	2.0 ± 0.3	8
Adrex (1 day)	48 hours	4.3 ± 0.9*	8
Sham (10 day)	48 hours	2.7 ± 0.2	9
Adrex (10 day)	48 hours	6.8 ± 1.1*	9

Rats were adrenalectomized or sham operated 1 or 10 days prior to challenge with phenobarbital (110 mg/kg, IP). Serum was collected at awakening or 48 hours after awakening. The time after phenobarbital administration is indicated.

Values are expressed as means ± SEM.

*$p<0.05$ Compared to corresponding sham operated group.

reflex. The replacement regimen was sufficient to restore glucocorticoid levels in adrenalectomized animals. Prior to replacement therapy with glucocorticoids, in this experiment, levels of corticosterone were undetectable in adrenalectomized rats whereas after chronic replacement, levels rose to 3.3±0.4 μg/100 ml (n=8). These were not different from levels measured in sham operated animals (3.2±0.5 μg/100 ml, n=6). As a physiological correlate of glucocorticoid state, thymus weights were also measured upon autopsy and as expected, were influenced by adrenalectomy and glucocorticoid replacement. Thymus weights of 10 day adrenalectomized rats were 591±38 mg (n=7) compared to 472±47 mg (n=6) of the sham operated group.

Chronic glucocorticoid administration to sham operated rats had no effect on the hypnotic response to phenobarbital (Table 2) despite the fact that corticosterone treatment tended to increase the corticosterone levels of sham operated rats from 3.2±0.5 μg/100 ml (n=6) to 7.0±2.3 μg/100 ml (n=8) and corticosterone and dexamethasone decreased thymus weights of sham operated rats to 420±59 mg (n=8) and 68±11 mg (n=8), respectively, compared to 472±47 mg (n=6) of controls.

In contrast to glucocorticoids, chronic treatment with the mineralocorticoids, aldosterone (0.25 mg, SC, on alternate days over the 10 day period) or 18-hydroxydeoxycorticosterone (0.5 mg, SC) on the same schedule did not antagonize the hypersensitivity of adrenalectomized animals to phenobarbital (Table 3). The absence of glucocorticoid activity in the preparations was confirmed by their inability to significantly depress thymus weights of adrenalectomized animals (665.3±35.1 mg, n=8, aldosterone-treated; 695.8±45.6 mg, n=8, 18-hydroxydeoxycorticosterone-treated).

Hypothermic Response to Phenobarbital

The degree of hypothermia in response to phenobarbital-induced hypnosis was monitored to assess the degree of hypersensitivity to the drug and to consider the possibility that impaired thermoregulatory mechanisms may contribute to the prolonged hypnotic response in adrenalectomized animals. There was no significant difference between adrenalectomized and sham operated rats in either basal body temperatures or the degree of phenobarbital-induced hypothermia during the duration of hypnosis. However, both acutely and chronically adrenalectomized rats were unable to regain normal body temperatures long after sham operated rats were euthermic (Fig. 1A, 1B). Acutely adrenalectomized rats were hypothermic as long as 10 hours after awakening from phenobarbital-induced narcosis while chronically adrenalectomized rats were hypothermic even at 24 hours. These animals felt cold to the touch and demonstrated behavioral and physiological responses such as huddling and piloerection but not shivering.

Adrenal catecholamines are rapidly released in response to cold-induced hypothermia [20] and are necessary for mobilization of energy stores in adjusting to this form of stress [23]. It was therefore possible that the absence of adrenomedullary catecholamines contributed to the prolonged hypothermia induced by phenobarbital in the adrenalectomized state. Rats adrenodemedullated 10 days prior to phenobarbital challenge did not respond any differently than sham operated controls to the hypothermic effects of phenobarbital (Fig. 1C), suggesting that adrenal steroids mediate the recovery from phenobarbital-induced hypothermia.

To test this hypothesis, 10 day adrenalectomized rats were administered glucocorticoid or mineralocorticoid replacements on a chronic basis to investigate the ability of these steroids to antagonize the prolonged hypothermia to phenobarbital. Administration of either corticosterone and dexamethasone completely restored the ability to regain normal body temperatures after phenobarbital-induced hypothermia in adrenalectomized animals (Fig. 2). In contrast, the mineralocorticoid, 18-hydroxydeoxycorticosterone, was completely ineffective while aldosterone transiently antagonized the prolonged barbiturate-induced hypothermia (Fig. 3).

We next considered the possibility that altered disposition may play a role in the increased sensitivity of adrenalectomized animals to the hypnotic and hypothermic effects of phenobarbital. Adrenalectomized rats awoke at serum levels of phenobarbital similar to those of intact rats but their times to awakening were 30–50% longer (Table 4) indicating that elimination of the drug was affected to some extent by glucocorticoids. However, it did not appear to be accumulating to any great extent in the adrenalectomized animal because at 10 hours after awakening, there were no differences between sham operated and adrenalectomized rats in serum levels of the drug (52 ± 9 μg/ml, n=4; 53 ± 6 μg/ml, n=4, respectively) despite the fact that the adrenalectomized rats were hypothermic. At 48 hours after awakening, adrenalectomized rats did have significantly higher levels of phenobarbital in serum but more than 90% of the drug had been eliminated from serum by this time point (Table 4).

DISCUSSION

Adrenalectomized rats were found to be more sensitive to the hypnotic effects of the barbiturates in agreement with numerous reports in the literature [16, 30, 38, 39, 44]. The results in this paper suggest that the hypersensitivity to phenobarbital may have a temporal component which appears to be related to the duration of the adrenalectomized state rather than the deficiency of circulating glucocorticoids. Rats adrenalectomized for 1 day showed a 30% increase in the duration of the loss of righting reflex when compared to sham operated controls. In rats adrenalectomized for 10 days, this effect was more pronounced.

Adrenalectomy also significantly decreased the time to onset of loss of righting reflex. This effect was consistently observed regardless of the duration of the adrenalectomized state and suggests that this component of the hypnotic response to phenobarbital is markedly sensitive to levels of circulating glucocorticoids. In fact, in one preliminary experiment, animals adrenalectomized only 3 hours prior to phenobarbital challenge already exhibited a significant reduction in the time to onset of loss of righting reflex (12.4 ± 1.5 min, n=8, sham; 7.4 ± 0.6 min, n=8, adrex, unpublished observations). Although corticosterone levels were not measured in this particular experiment, it has been reported that by 2.5 hours following adrenalectomy, corticosterone, which has a half life of 20 minutes in the rat [32] is depleted 95% [4].

This hypersensitivity of adrenalectomized animals to the barbiturates may result from alterations in central nervous system processes or alterations in disposition. In support of the former hypothesis, work done in this laboratory has shown that adrenalectomized rats administered phenobarbital by the intracerebroventricular route, which circumvents the contribution of the liver to drug metabolism, become progressively more sensitive to repeated doses of the drug. Sham operated animals, on the other hand, become tolerant to the drug administered by this route (Villano *et al.*, in preparation). It is possible that glucocorticoid deficiency has affected neurotransmitter function thought to be involved in barbiturate action [28,29]. For instance, adrenalectomy has been reported to increase uptake of GABA in at least one brain area, the hippocampus [26], and to decrease serotonin turnover in the same area [40]. Intact serotonergic function is necessary for tolerance development to phenobarbital [22].

On the other hand, the data presented here suggest that altered pharmacokinetic disposition plays a role in the hypersensitivity to peripheral administration of the drug. Blood levels of phenobarbital determined at emergence from anesthesia indicated no difference between adrenalectomized and sham operated groups. However, adrenalectomized animals were awakening 30 to 60% later than controls suggesting that differences in disposition are present.

Levels of brain phenobarbital were not determined in this study but are assumed to be in equilibrium with levels in the serum, as described previously [11]. Recently, Long and Holaday [21] presented evidence that there is increased permeability of the blood brain barrier of adrenalectomized rats to ^{125}I-bovine serum albumin suggesting that glucocorticoids may indirectly increase uptake of substances in the CNS. In view of this, the distribution kinetics of phenobarbital in the brains of adrenalectomized animals should be reexamined. Because we did not measure brain phenobarbital levels in this study, we cannot exclude a centrally mediated mechanism of hypersensitivity to phenobarbital-induced hypnosis.

In agreement with the reports that glucocorticoids are effective in reducing sensitivity to hexobarbital [30], pentobarbital [6,9] and barbital [16] in adrenalectomized

animals, corticosterone and dexamethasone were effective in shortening the duration of the hypnotic response to phenobarbital in 10 day adrenalectomized rats. There was also a tendency for these compounds to delay the times to onset of loss of righting reflex although these values were not statistically significant.

In contrast, neither aldosterone, the principle mineralocorticoid of the rat [5], not 18-hydroxydeoxycorticosterone, a weak mineralocorticoid [13,42] secreted in abundance by the rat adrenal [10], altered the hypnotic response to phenobarbital following adrenalectomy. Moreover, adrenodemedullation, with sufficient time allowed for regeneration of the adrenal cortex, was without effect in altering the hypnotic response to phenobarbital providing further evidence that the hypersensitivity following adrenalectomy was caused by glucocorticoid deficiency.

Dexamethasone failed to affect the hypnotic response of sham operated rats despite the fact that its potency was sufficient to reduce thymus weights 85%. Likewise, corticosterone administration, which also caused thymus gland involution (10%) and increased serum corticosterone levels, did not change the hypnotic response of sham operated animals to phenobarbital. This is in contrast to the findings of Komiya and Shibata [17] who reported that either hydrocortisone, cortisone or ACTH could decrease the duration of the hypnotic response to intravenously administered barbital in intact mice in doses that were lower in glucocorticoid potency than the doses used in this study. Differences in the species of animal, housing conditions, the barbiturate or the route of administration of the barbiturate may account for these differences.

In preliminary experiments designed to examine the ability of adrenalectomized rats to develop tolerance to repeated doses of phenobarbital, it became apparent that adrenalectomized rats frequently succumbed to nonhypnotic doses of intraperitoneally administered drug (40 mg/kg, 2× daily for 5 days) that were nontoxic to sham operated rats. There was also a temporal component to this increased toxicity. In a limited study, 40% of animals adrenalectomized one day prior to the start of a chronic phenobarbital regimen expired by the fifth day of the regimen. On the other hand, 80% of chronically adrenalectomized rats (10 days) succumbed by the fifth day of the chronic phenobarbital treatment (unpublished observations). Whether accumulation of the drug occurs upon repeated administration due to slower elimination remains to be determined. However this is unlikely because by 48 hours after awakening, serum phenobarbital levels of sham and adrenalectomized rats were less than 3% and 7% respectively, of those measured at awakening. On the other hand, adrenalectomized rats showed piloerection, huddling and felt cold to the touch in addition to appearing sedated. For this reason, the hypothermic response to phenobarbital was investigated as a possible factor contributing to toxicity to phenobarbital following adrenalectomy.

In agreement with others, adrenalectomy per se did not affect the basal body temperature [37], but it did markedly alter the ability of these animals to recover normal body temperatures after phenobarbital administration. Prolonged hypothermia lasting for 10 hours or more after awakening from phenobarbital-induced hypnosis was manifest in both 1 and 10 day adrenalectomized animals whereas sham operated animals were euthermic by 4 hours after recovery of the righting reflex. The magnitude of the maximum fall in body temperature was the same for both adrenalectomized and sham animals. Accumulation of the drug cannot account for the prolonged hypothermia because at 10 hours after awakening, both sham and adrenalectomized animals have the same serum phenobarbital levels (52 ± 9 μg/ml and 53 ± 6 μg/ml, respectively) and the adrenalectomized animals are hypothermic while the sham operated are not.

Regulation of body temperature [12,37], which is disrupted by adrenalectomy, depends on a complex relationship between behavioral responses, i.e., shivering, peripheral utilization of fuel, and central regulation via hypothalamic thermostats. Glucocorticoids have no direct effect in themselves on the basal metabolic rate [37], but they are believed to combat cold-induced hypothermia by restoring metabolic mechanisms of thermogenesis such as free fatty acid mobilization and utilization of glucose [24]. In the absence of glucocorticoids, the lipolytic and glycolytic responses to epinephrine are blunted [33]. As a consequence, adrenalectomized animals are unable to raise body temperatures above ambient levels after they are removed from a cold environment [41]. Thus they respond to phenobarbital-induced hypothermia in the same way as they do to cold-induced hypothermia. The key role of the glucocorticoids in thermoregulation is further supported by the fact that the glucocorticoids, dexamethasone and corticosterone, were highly effective in preventing the prolonged phenobarbital-induced hypothermia in adrenalectomized animals, analogous to their ability to restore thermoregulatory responses in adrenalectomized animals exposed to cold stress [24].

Aldosterone was also effective in averting the prolonged phenobarbital-induced hypothermia, albeit transiently. The animals were again hypothermic at 10 hours after awakening from the anesthesia. Although it is possible that at this dose aldosterone may have had some glucocorticoid-like activity, it is unlikely in view of the lack of effect on thymus weights. On the other hand, it has been reported that aldosterone and corticosterone bind to the same subpopulation of receptors in the brain [2].

Unlike the report that chronically adrenodemedullated animals are unable to maintain body temperatures in a cold environment [23], 10 day adrenodemedullated and sham operated rats were no different in their responses to phenobarbital-induced hypothermia. One explanation is that different mechanisms of thermoregulation may be involved in combating cold-induced hypothermia as compared to phenobarbital-induced hypothermia. Phenobarbital-induced hypothermia may be a less severe form of cold stress and adaptation to it is independent of secretion from the adrenal medulla.

The mechanisms by which the barbiturates induce hypothermia are not well understood. A number of studies have emphasized the depressant effects of the barbiturates on metabolic functions such as O_2 consumption [7,36] and glucose utilization [1,43]. On the other hand, hypothermia develops following barbiturate withdrawal in dependent mice [3] suggesting that these drugs also have direct effects on central thermoregulatory centers.

Glucocorticoids may also modulate thermoregulation partially through a central mechanism. Chowers *et al.* [8] reported that cortisol applied directly to the preoptic area prevents the febrile response to pyrogen in rabbits. If central mechanisms are involved in phenobarbital-induced hypothermia, then direct effects of glucocorticoids on thermoregulatory centers, i.e., to reset central thermostats, may explain the reversal of the hypothermia. However, adrenalectomized rats administered a hypnotic dose of phenobarbital by the ICV route, do not exhibit the prolonged

hypothermia characteristic of adrenalectomized animals administered the drug intraperitoneally (Villano *et al.*, in preparation) which argues against a centrally mediated mechanism. These two studies may not be completely comparable because rats receiving ICV phenobarbital (800 μg) do not sleep as long as rats administered a hypnotic dose (110 mg/kg) intraperitoneally. In order to identify peripheral contributions of this response, more detailed comparisons between normal and glucocorticoid deficient animals in their metabolic responses, e.g., thyroid hormone levels, to phenobarbital are necessary.

In summary, these studies indicate that adrenalectomized animals are more sensitive to the hypothermic as well as the hypnotic effects of phenobarbital, that glucocorticoids are effective in antagonizing this sensitivity, and suggest that prolonged hypothermic responses may be responsible for the increased toxicity to the barbiturates observed in glucocorticoid deficiency.

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REFERENCES

1. Astrup, J., P. M. Sorensen and H. R. Sorensen. Inhibition of cerebral oxygen and glucose consumption in the dog by hypothermia, pentobarbital, and lidocaine. *Anesthesiology* **55:** 263–268, 1981.
2. Beaumont, K. and D. D. Fanestil. Characterization of rat brain aldosterone receptors reveals high affinity for corticosterone. *Endocrinology* **113:** 2043–2051, 1983.
3. Belknap, J. K. and M. A. Mitchell. Barbiturate physical dependence in mice: effects on body temperature regulation. *J Pharmacol Exp Ther* **218:** 647–652, 1981.
4. Buckingham, J. C. and J. R. Hodges. Interrelationships of pituitary and plasma corticotrophin and plasma corticosterone in adrenalectomized and stressed, adrenalectomized rats. *J Endocrinol* **63:** 213–222, 1974.
5. Bush, I. E. Species differences in adrenocortical secretion. *J Endocrinol* **9:** 95–100, 1953.
6. Chambers, W. F., S. L. Freedman and C. H. Sawyer. The effect of adrenal steroids on evoked reticular responses. *Exp Neurol* **8:** 458–469, 1963.
7. Chance, B. and G. Hollunger. Inhibition of electron and energy transfer in mitochondria. I. Effects of amytal, thiopental, rotenone, progesterone and methylene glycol. *J Biol Chem* **238:** 418–431, 1963.
8. Chowers, I., N. Conforti and S. Feldman. Local effects of cortisol in the preoptic area on temperature regulation. *Am J Physiol* **214:** 538–542, 1968.
9. Cook, S. and H. Mavor and W. F. Chambers. Effects of reticular stimulation in altered adrenal states. *EEG Clin Neurophysiol* **12:** 601–608, 1960.
10. Cortes, J. M., F. G. Peron and R. I. Dorfman. Secretion of 18-hydroxydeoxycorticosterone by the adrenal gland. *Endocrinology* **73:** 713–720, 1963.
11. Danhof, M. and G. Levy. Kinetics of drug action in disease states. 1. Effect of infusion rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. *J Pharmacol Exp Ther* **229:** 44–50, 1984.
12. Deavers, D. R. and X. J. Musacchia. The function of glucocorticoids in thermogenesis. *Fed Proc* **38:** 2177–2181, 1979.
13. Feldman, D. and J. W. Funder. The binding of 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone to mineralocorticoid and glucocorticoid receptors in the rat kidney. *Endocrinology* **92:** 1389–1396, 1973.
14. Ho, I. K. Systematic assessment of tolerance to pentobarbital by pellet implantation. *J Pharmacol Exp Ther* **197:** 479–487, 1976.
15. Kato, R. and J. R. Gillette. Sex differences in the effects of abnormal physiological states on the metabolism of drugs by rat liver microsomes. *J Pharmacol Exp Ther* **150:** 285–291, 1965.
16. Komiya, A. and K. Shibata. Effect of adrenalectomy and replacement with adrenocortical steroids on barbital anesthesia in mice. *J Pharmacol Exp Ther* **116:** 98–106, 1956.
17. Komiya, A. and K. Shibata. Effect of adrenocortical steroids and ACTH administration on barbital anesthesia in normal mice. *J Pharmacol Exp Ther* **117:** 68–74, 1956.
18. Kramer, C. Y. Extension of multiple range tests to groups with unequal numbers of replications. *Biometrics* **12:** 307–310, 1956.
19. Krey, L. C., K.-H. Lu, W. R. Butler, W. R. Hotchkiss, F. Piva and E. Knobil. Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. II. GH and cortisol secretion. *Endocrinology* **96:** 1088–1093, 1975.
20. Leduc, J. Catecholamine production and release in exposure and acclimation to cold. *Acta Physiol Scand* **53:** Suppl 183, 1–101, 1961.
21. Long, J. B. and J. W. Holaday. Blood-brain barrier: endogenous modulation by adrenal-cortical function. *Science* **227:** 1580-1582, 1985.
22. Lyness, W. H. and M. J. Mycek. The role of cerebral serotonin in the development of tolerance to centrally administered phenobarbital. *Brain Res* **187:** 443–456, 1980.
23. Maickel, R. P., N. Matussek, D. N. Stern and B. B. Brodie. The sympathetic nervous system as a homeostatic mechanism. I. Absolute need for sympathetic nervous function in body temperature maintenance of cold-exposed rats. *J Pharmacol Exp Ther* **157:** 103–110, 1967.
24. Maickel, R. P., D. N. Stern, E. Takabatake and B. B. Brodie. The sympathetic nervous system as a homeostatic mechanism. II. Effect of adrenocortical hormones on body temperature maintenance of cold-exposed adrenalectomized rats. *J Pharmacol Exp Ther* **157:** 111–116, 1967.
25. Maynert, E. W. and H. B. Van Dyke. The absence of localization of barbital in divisions of the central nervous system. *J Pharmacol Exp Ther* **98:** 184–187, 1950.
26. Miller, A. L., C. Chaptal, B. S. McEwen and E. J. Peck, Jr. Modulation of high affinity GABA uptake into hippocampal synaptosomes by glucocorticoids. *Psychoneuroendocrinology* **3:** 155–164, 1978.
27. Mycek, M. J. and H. Brezenoff. Tolerance to centrally administered phenobarbital. *Biochem Pharmacol* **25:** 501–504, 1976.
28. Nicoll, R. A. Pentobarbital: Differential postsynaptic actions on sympathetic ganglion cells. *Science* **199:** 451–452, 1978.
29. Ransom, B. R. and J. L. Barker. Pentobarbital selectively enhances GABA-mediated postsynaptic inhibition in tissue cultured mouse spinal neurons. *Brain Res* **114:** 530–535, 1976.
30. Remmer, H. Induction of drug metabolizing enzyme system in the liver. *Eur J Clin Pharmacol* **5:** 116–136, 1972.
31. Robillard, E., A. D'Iorio and J. Pellerin. Influences endocriniennes sur la desintoxication hepatique du pentobarbital. *Union Med Can* **83:** 853–860, 1954.
32. Schapiro, S., C. J. Percin and F. J. Kotichas. Half-life of plasma corticosterone during development. *Endocrinology* **89:** 284–286, 1971.
33. Shafrir, E., K. E. Sussman and D. Steinberg. Role of the pituitary and the adrenal in the mobilization of free fatty acids and lipoproteins. *J Lipid Res* **1:** 459–465, 1960.
34. Spector, S. and E. J. Flynn. Barbiturates: radioimmunoassay. *Science* **174:** 1036–1038, 1971.

35. Steelman, S. L. and R. Hirschmann. Synthetic analogs of the adrenal cortical steroids. In: *The Adrenal Cortex*, edited by A. B. Eisenstein. Boston: Little, Brown and Co., 1967, pp. 345–383.
36. Steen, P. A., L. Newberg, J. H. Milde and J. D. Michenfelder. Hypothermia and barbiturates: individual and combined effects on canine cerebral oxygen consumption. *Anesthesiology* **58:** 527–532, 1983.
37. Tanache, M. The adrenal gland and thermoregulation. *Israel J Med Sci* **12:** 1019–1025, 1976.
38. Tureman, J. R., W. M. Booker and C. Froix. Response of adrenalectomized rats to pentobarbital sodium. *Am J Physiol* **167:** 833, 1951.
39. Turemann, J. R., A. N. Maloney, W. M. Booker, C. Froix and W. Jones. Further studies on one depressant action of pentobarbital sodium on adrenalectomized rats. *J Pharmacol Exp Ther* **106:** 420, 1952.
40. Van Loon, G. R., A. Shum and M. J. Sole. Decreased brain serotonin turnover after short term (two hour) adrenalectomy in rats: a comparison of four turnover methods. *Endocrinology* **108:** 1392–1402, 1981.
41. Vidovic, V. L. and V. Popovic. Studies on the adrenal and thyroid glands of the ground squirrel during hibernation. *J Endocrinol* **11:** 125–133, 1954.
42. Ward, P. J. and M. K. Birmingham. Properties of ultraviolet-absorbing lipids produced by rat adrenals in vitro. *Biochem J* **76:** 269–279, 1960.
43. Webb, J. L. and K. A. C. Elliott. Effects of narcotics and convulsants on tissue glycolysis and respiration. *J Pharmacol Exp Ther* **103:** 24–34, 1951.
44. Yanai, J. and P. Y. Sze. Adrenal glucocorticoids as a required factor in barbiturate-induced changes in functional tolerance and brainstem tryptophan hydroxylase. *Brain Res* **269:** 297–302, 1983.