Effects of Urethane on Pituitary–Adrenal Function in the Rat[1] (37436)

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Although urethane anesthesia is frequently used in studies concerned with testing of neuronal responsiveness to hormone administration (1–7), little is known about the possibility of direct effects of urethane on neuroendocrine function. Only recently (5, 8–10) has the influence of urethane on gonadotropic function been examined; little information is available concerning its actions on the adrenal cortex (11).

Studies in our laboratory have attempted to correlate hypothalamic electrical activity with pituitary–adrenal function in rats anesthetized with urethane (6). The present work performed in conjunction with these electrophysiological studies provides further information concerning the pituitary–adrenal activity during urethane anesthesia. In particular, data were obtained about feedback suppression of ACTH release in response to dexamethasone, and on ACTH secretion following various stimuli.

Materials and Methods. Experiments were performed on male Sprague–Dawley rats weighing 225–300 g. They were maintained at constant temperature (23 ± 0.5°) with an alternating light–dark schedule (0600 to 1800 lights on) and given food pellets (Purina Lab Chow) and water *ad libitum*. Rats were anesthetized with urethane (1.25 g/kg body wt) administered intraperitoneally.

Drugs were dissolved in 0.9% saline, and unless otherwise noted, administered through a jugular cannula in the following doses (calculated per 100 g body wt): dexamethasone 21-phosphate, 10, 20, 80 μg; histamine diphosphate, 2000 μg free base; vasopressin, 100 mU; insulin, 0.4 U; ACTH, 50 mU. Formalin was given subcutaneously at a dose of 1 ml of a 10% solution/animal. Serial blood samples were taken with one or two microhematocrit tubes (60–120 μl) from a small incision in the tail. The plasma was separated promptly and frozen. Plasma corticosterone levels were determined subsequently by the competitive protein-binding method of Murphy (12). Dexamethasone, when administered, was given twice, 2 and 3 hr after anesthesia. Other drugs were administered 4 hr after the first dexamethasone injection and blood samples were drawn 15, 30 and 60 min later. ACTH was always administered in the last time period for each study and blood was drawn 15 and 30 min thereafter.

Data were analyzed using Student's t test for paired observations. In every case, each animal served as its own control. The mean difference ($\bar{D}$) was generated with its standard error ($SE_{\bar{D}}$), and used to calculate t. Unless otherwise stated, plasma corticosterone levels in all tables and figures are depicted as mean difference ($\bar{D}$) ± $SE_{\bar{D}}$.

Results. Plasma concentrations of corticosterone (B) were markedly elevated from a base line of 7.0 ± 1.9 μg/ml (mean ± SE) to 55.4 ± 4.3, 53.4 ± 6.2, and 49.4 ± 2.8 at 2–4 min, 1, 2, and 4 hr, respectively, after urethane injection. Animals injected with saline had comparatively low B levels, 1.4 ± 0.7, by 2 hr. ACTH administered produced no further alteration in plasma B levels in the urethane-anesthetized animals indicating that the maximal secretory capacity of the adrenal

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TABLE I. The Effect of Dexamethasone on Plasma B Levels (μg/100 ml) in Rats Anesthetized with Urethane.

Group	Dexamethasone dose (μg/100 g)[a]	No.	Initial B level	Plasma B change at 4 hr[b]
Intact	0	4	55.4	— 6.2 ± 7.8
	10	5	53.2	—35.7 ± 2.8[c]
	20	24	52.2	—39.8 ± 2.5[c]
	80	4	54.4	—43.7 ± 5.0[c]

[a] Each dose of dexamethasone was injected twice per animal.

[b] Values are mean differences ($\bar{D}$) ± $SE_{\bar{D}}$ generated from the initial plasma B level.

[c] Differs from initial B level at $p < 0.01$.

had already been obtained.

Dexamethasone was administered after urethane anesthesia and plasma B concentrations were measured at hourly intervals. Intact rats given doses of 10, 20, or 80 μg/100 g body weight all showed decreased levels of corticosterone at 4 hr consistent with depression of ACTH release (Table I).

The effects of single doses of various compounds on plasma B levels in animals suppressed with dexamethasone are shown in Table II. The values represent changes in plasma B produced by each stimulus with reference to the four hour dexamethasone level. The last two columns in Table II represent plasma B increments after ACTH injection. These are also calculated from the 4-hr dexamethasone level to allow comparison of ACTH-induced and stress-induced increments. All agents stimulated pituitary ACTH release; saline, the vehicle control, was ineffective. Generally, each stimulus resulted in sustained elevation of plasma B throughout the 1-hr observation period. Only the effects of vasopressin began to subside at the end of the hour. Histamine was also administered to two groups suppressed with different doses (10 or 80 μg/100 g) of dexamethasone. No alteration in response pattern or in absolute increments to histamine was observed over the range in amount of dexamethasone administered. Peak plasma B concentration was attained within 15 min and persisted thereafter. The B level in all groups appeared to be nearly maximal as indicated by comparison with the increment after ACTH administration.

The response to insulin is illustrated in detail in Fig. 1, which also depicts the hourly changes in plasma B concentration following pretreatment with dexamethasone. Plasma B levels fell progressively for 4 hr. Insulin injected at that time produced a significant increment within 15 min with a continued rise to a maximal level at 30 min. ACTH administration at the end of the experiment elicited a small additional increment in plasma B at 30 min ($p < 0.05$).

The high plasma B levels in rats anesthetized with urethane were lowered within 2 hr by hypophysectomy (2.3 ± 0.9), with no further change occurring by 4 hr (2.1 ± 0.6) (mean ± SE). Histamine, formalin, or vasopressin administered at the same dose levels used previously did not alter the plasma B concentrations in these hypophysectomized animals. However, a highly significant response to ACTH was obtained in each instance.

Discussion. Administration of urethane results in enhanced secretion of ACTH as shown by elevated plasma levels of corticosterone (B). This confirms the only previous investigation on the effects of urethane on pituitary–adrenal function conducted by Spriggs and Stockham (11). In addition, our data indicate that urethane does not alter plasma B concentrations in hypophysectomized rats, suggesting that it has no direct effect on the adrenal gland in the absense of ACTH.

Dexamethasone was used to inhibit endogenous ACTH secretion, and, in rats given urethane plasma B levels were lowered to 10–13 μg/100 ml. Such levels are slightly higher than those in resting animals or in animals 4

TABLE II. Changes in Plasma B Levels (μg/100 ml) Produced by Various Stimuli in Intact, Urethane-Anesthetized Rats Pretreated 4 hr Previously with Dexamethasone.[e]

Stimulus	No.	Time after stimulus (min)			Time after ACTH (min)	
		15	30	60	15	30
Saline	6	$-$ 4.2 $\pm$ 2.7	$-$ 7.6 $\pm$ 2.9	$-$ 3.0 $\pm$ 3.2	+26.1 $\pm$ 8.4[ac]	+31.6 $\pm$ 8.6[ac]
Histamine	3	+33.0 $\pm$ 7.3[a]	+31.5 $\pm$ 5.5[a]	+28.4 $\pm$ 3.5[a]	—	—
Histamine[f]	5	+24.1 $\pm$ 2.3[b]	+20.8 $\pm$ 6.5[ad]	+25.8 $\pm$ 6.9[ad]	+30.6 $\pm$ 2.8[b]	+33.9 $\pm$ 4.0[a]
Histamine[g]	4	+27.4 $\pm$ 3.7[b]	+31.2 $\pm$ 3.8[b]	+30.5 $\pm$ 3.5[b]	+37.2 $\pm$ 2.6[b]	+35.8 $\pm$ 4.0[b]
Insulin	6	+18.2 $\pm$ 2.0[b]	+27.0 $\pm$ 3.2[b]	+26.6 $\pm$ 5.3[b]	+34.3 $\pm$ 4.9[b]	+37.7 $\pm$ 5.0[b]
Formalin	4	+21.3 $\pm$ 2.3[b]	+26.4 $\pm$ 2.6[b]	+32.7 $\pm$ 1.0[b]	+29.9 $\pm$ 3.4[bc]	+32.9 $\pm$ 5.8[bc]
Vasopressin	5	+28.5 $\pm$ 1.1[b]	+33.8 $\pm$ 3.1[b]	+14.7 $\pm$ 3.4[a]	+39.5 $\pm$ 2.2[bc]	+45.0 $\pm$ 3.5[bc]

[a] Differs from 4 hr post-dexamethasone level at $p < 0.05$.

[b] Differs from 4 hr post-dexamethasone level at $p < 0.01$.

[c] Group size reduced to 3 rats.

[d] Group size reduced to 4 rats.

[e] All values are mean differences ($\bar{D}$) $\pm$ $SE_{\bar{D}}$ generated by comparison with the plasma B level at 4 hr after dexamethasone. Dexamethasone dose was 20 μg/100 g body weight except as noted.

[f] Dexamethasone dose 10 μg/100 g.

[g] Dexamethasone dose 80 μg/100 g.

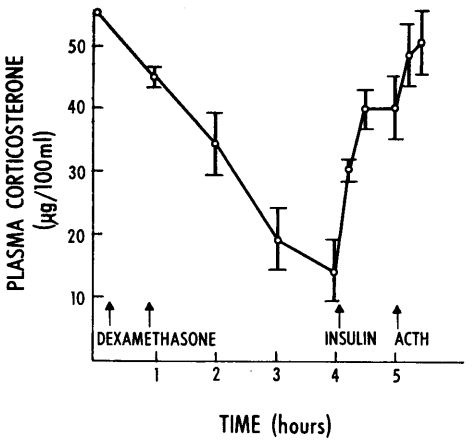

FIG. 1. Effects of dexamethasone and insulin on plasma corticosterone concentrations in intact rats anesthetized with urethane. Each point represents the mean of 6 values. Verticle bars indicate ± SE$\bar{D}$.

hr after hypophysectomy. Based on other reports, dexamethasone suppression of ACTH secretion at 2–4 hr is usually complete (13–15), suggesting that urethane-anesthetized animals in the present study did not respond completely to negative feedback. The basis for this effect may involve several factors including alterations in the sensitivity of feedback receptors to dexamethasone induced by urethane.

All stimuli used were effective in eliciting ACTH release in urethanized rats despite high doses of dexamethasone and only the response to saline was abolished. Thus, the urethane preparation is similar to other intact preparations in that high doses of dexamethasone are ineffective in blocking the release of ACTH by such stimuli (14, 16). Histamine, for example, produced a sustained elevation in the plasma B levels which was unaltered by an 8-fold increase in the dose of dexamethasone. Increments in plasma B levels in dexamethasone-treated rats were also observed with insulin, formalin, and vasopressin. Direct action by these stimuli on the adrenal gland was excluded by their total ineffectiveness in hypophysectomized rats. Thus, urethane anesthesia does not abolish the ACTH release induced by certain traumatic stimuli in dexamethasone-suppressed animals.

The importance of these observations is threefold: Firstly, since urethane is a commonly employed anesthetic in studies involving recording of neural responses in the central nervous system, it is of interest that its administration elevates blood corticosterone levels during the time period when most electrical recording studies are performed. Secondly, administration of dexamethasone lowers endogenous corticosterone levels in the presence of urethane anesthesia, although the response is apparentiy attenuated. Finally, several agents known to activate the hypothalamic–pituitary–adrenal system result in marked breakthrough stimulation of adrenal secretion when administered to dexamethasone-suppressed, urethane-anesthetized rats.

Summary. Urethane anesthesia produces elevated plasma concentrations of corticosterone (B) that are sustained over a 4-hour observation period. The elevated B levels are reduced after intravenous administration of dexamethasone (20 μg/100 ml body weight) within 3–4 hr, although the steroid suppression of ACTH release was not complete. Histamine (2000 mg), insulin (0.4 U), formalin (1 ml of 10% solution) and vasopressin (100 mU) each elicited an increase in plasma corticosterone levels in dexamethasone-pretreated rats. No increments were observed after administration of the same agents in hypophysectomized rats.

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