EFFECT OF CHLORPROMAZINE ON PITUITARY-ADRENAL FUNCTION IN THE DOG

By
Donna Betz and William F. Ganong

ABSTRACT
The response of the adrenal cortex to psychic and surgical stress and to exogenous ACTH has been investigated in dogs following the acute and chronic administration of chlorpromazine. Administration of 2.5 mg/kg intravenously followed by 2.5 mg/kg over a 1/2-hour period to stressed dogs did not decrease adrenal 17-hydroxycorticoid output significantly, while surgical hypophysectomy caused a marked drop in the same time interval. In dogs given 30 mg/kg/day, the rise in peripheral plasma 17-hydroxycorticoids following 2 hours of immobilization was normal after 3 days of treatment, and the response to surgical stress and to 1 IU of ACTH was normal in dogs treated for 5 days. After hypophysectomy, 17-hydroxycorticoid output in chlorpromazine-treated dogs in response to 2, 5, and 10 mU doses of ACTH was slightly greater than normal, probably due to the mild adrenal hypertrophy produced by the drug. Chlorpromazine does not interfere with the determination of 17-hydroxycorticoids by the Silber-Porter method.

Chlorpromazine has been reported to have a variety of actions on the hypothalamo-pituitary-adrenocortical system (Ganong & Forsham 1960). Some experiments suggest that the drug increases ACTH secretion, others that it inhibits the increase in ACTH secretion produced by various stresses, and still others that it inhibits the adrenocortical response to ACTH. These conflicting results may be due in part to differences in the doses of chlorpromazine used, and to differences in the animal species studied, but many of the studies

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264
employed such indirect indicators of adrenocortical activity as adrenal ascorbic acid depletion in the rat, and urinary steroid excretion. In the present experiments, the effect of chlorpromazine on pituitary-adrenal function in the dog has been studied using peripheral plasma levels and adrenal venous outputs of 17-hydroxycorticoids as well as adrenal weights as the indices of adrenocortical function. Part of this data has previously been published in an abstract (Gannong & Betz 1960).

**METHODS**

In 6 pentobarbital anaesthetized mongrel dogs, the right adrenal vein was cannulated by the method of Hume & Nelson (1955). The dogs were then given a single dose of 2.5 mg/kg of chlorpromazine intravenously, followed by 2.5 mg/kg by constant infusion in 50 ml of isotonic saline over a 30-minute period. 17-hydroxycorticoid output was measured in timed samples of adrenal vein blood before and immediately after the infusion. In 3 control dogs, the right adrenal vein was cannulated and 17-hydroxycorticoid output determined before and 30 minutes after surgical hypophysectomy.

In another experiment, 10 healthy male mongrel dogs weighing 9.0–17.2 kg were given 10 mg/kg of chlorpromazine intramuscularly 3 times a day. Four control dogs were given similarly timed saline injections. On the third day of treatment, the control dogs and 7 of the chlorpromazine-treated dogs were immobilized in a canvas sling stand for 2 hours (Gannong 1961). Peripheral arterial blood samples were collected immediately before and after the period of immobilization.

On the fifth day of treatment, the control dogs and 8 of the chlorpromazine-treated dogs were subjected to operative stress by cannulation of the right adrenal vein under pentobarbital anaesthesia and a sample of adrenal venous blood collected. In most instances, 1 IU of ACTH (Upjohn) was then injected intravenously, and adrenal venous blood collected starting 4 minutes after the ACTH injection. At autopsy, the right adrenal gland of each dog was removed and weighed, fixed in formalin, sectioned, and stained with eosin and haematoxylin for histological examination.

Before they were sacrificed, 4 of the chlorpromazine-treated dogs were hypophysectomized and their sensitivity to ACTH tested. Adrenal venous blood samples were collected 1/2 and 1 hour after hypophysectomy. ACTH was then injected in 2, 5, and 10 mU doses. The interval between injections was 30 minutes, and adrenal venous blood was collected from the fourth to the fourteenth minute after each injection (Nelson & Hume 1955). Six untreated hypophysectomized control dogs were similarly studied.

Adrenal venous and peripheral arterial blood samples were centrifuged promptly and the plasma stored in the frozen state for subsequent analysis of 17-hydroxycorticoid concentration by the method of Silber & Porter (1934). 17-hydroxycorticoid output was calculated by multiplying the 17-hydroxycorticoid concentration of each adrenal venous sample by the adrenal venous plasma flow per minute. To determine whether chlorpromazine interferes with the determination of 17-hydroxycorticoids by the Silber-Porter method, peripheral plasma from a normal dog was divided into eight 15 ml aliquots. 3 µg of cortisol were added to 4 of these aliquots, and 0.5 mg chlorpromazine added to 2 of the aliquots containing added cortisol and 2 to which cortisol had not been added. The content of 17-hydroxycorticoids in these samples was then determined by the Silber-Porter method.
RESULTS

Table 1 shows the 17-hydroxycorticoid values obtained after adding cortisol with and without chlorpromazine to dog peripheral plasma. It is apparent that the drug did not interfere with the method for measuring 17-hydroxycorticoids.

The acute administration of 5 mg/kg of chlorpromazine over a 30-minute period did not alter the adrenocortical response of the surgically stressed dogs. Before chlorpromazine, the mean 17-hydroxycorticoid output was 8.2 ± 1.2 µg/min (mean ± standard error). At the end of the 30-minute period, it had decreased insignificantly to 7.1 ± 0.7 µg/min. The mean 17-hydroxycorticoid output was 7.1 ± 1.9 µg/min before surgical hypophysectomy in the 3 dogs subjected to this operation, and 1.9 ± 0.9 µg/min 1/2 hour after the pituitary gland was removed.

The effects of chronic administration of 30 mg/kg/day of chlorpromazine on the adrenocortical response to emotional and surgical stress are summarized in Table 2. This dose produced considerable ataxia. The injected animals lay quietly in their cages most of the time, but when immobilized, they barked and whined in the same fashion as the controls. The mean peripheral 17-hydroxycorticoid level before immobilization was slightly but not significantly lower in the chlorpromazine-treated dogs, and the rise in peripheral 17-hydroxycorticoids produced by immobilization was similar to that in the saline-treated controls. The response to surgical trauma was slightly, although not significantly, greater in the chlorpromazine-treated dogs than it was in the controls, as was the response to 1 IU of ACTH.

17-hydroxycorticoid output fell to low levels within 1/2 hour after hypophys-

<table>
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<tr>
<th>Added chlorpromazine</th>
<th>Added cortisol</th>
<th>µg 17-OH-cort./sample</th>
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<tr>
<td>15 ml plasma</td>
<td>—</td>
<td>—</td>
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<tr>
<td>0.5 mg</td>
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<tr>
<td>0.5 mg</td>
<td>3 µg</td>
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<td>0.5 mg</td>
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Table 1.
Recovery of added cortisol free alcohol in peripheral dog plasma with and without added chlorpromazine.

266
Table 2.
Effect of chlorpromazine on pituitary adrenal function in the dog. Values are means ± standard errors. The figures in parentheses are numbers of animals.

<table>
<thead>
<tr>
<th></th>
<th>Chlorpromazine treated</th>
<th>Saline treated</th>
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<tbody>
<tr>
<td>Peripheral 17-hydroxycorticoids (μg %)</td>
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<td></td>
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<tr>
<td>A. Control</td>
<td>2.7 ± 1.0 (7)</td>
<td>4.3 ± 1.4 (4)</td>
</tr>
<tr>
<td>B. After 2 h of immobilization</td>
<td>15.1 ± 2.4 (7)</td>
<td>15.5 ± 2.1 (4)</td>
</tr>
<tr>
<td>17-hydroxycorticoid output from adrenal (μg/min)</td>
<td></td>
<td></td>
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<tr>
<td>A. After surgical trauma</td>
<td>9.1 ± 1.2 (8)</td>
<td>7.9 ± 0.7 (4)</td>
</tr>
<tr>
<td>B. After 1 IU ACTH (Upjohn)</td>
<td>13.5 ± 2.4 (5)</td>
<td>9.1 ± 1.5 (4)</td>
</tr>
<tr>
<td>Right adrenal weight (mg/kg)</td>
<td>66.8 ± 3.9 (8)</td>
<td>53.6 ± 8.7 (4)</td>
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Table 3.
Response to ACTH in chlorpromazine-treated and control hypophysectomized dogs. Values are means ± standard errors. Figures in parentheses are numbers of dogs.

<table>
<thead>
<tr>
<th></th>
<th>17-hydroxycorticoid output (μg/min)</th>
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<tr>
<td></td>
<td>1/2 h after hyp.</td>
<td>1 h after hyp.</td>
</tr>
<tr>
<td></td>
<td>2 mU</td>
<td>5 mU</td>
</tr>
<tr>
<td>Chlorpromazine-treated dogs</td>
<td>0.8 (2)</td>
<td>0.3 (3)</td>
</tr>
<tr>
<td>Untreated dogs</td>
<td>0.5 ± 0.02 (6)</td>
<td>1.5 ± 0.03 (5)</td>
</tr>
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Hyphoectomy in the chlorpromazine-treated dogs (Table 3). The response to 2, 5, and 10 mU doses of ACTH was greater in these dogs than it was in the controls, although the difference was statistically significant ($P < 0.01$) only at the 2 mU dose.

The mean weight of the right adrenals in the chlorpromazine-treated dog was slightly greater than that of the controls (Table 2). The difference was not statistically significant, but histologically, there was definite hypertrophy of the zona fasciculata in the treated dogs, while there was none in the controls.
EGDAHL & RICHARDS (1956) found that chlorpromazine increases the secretion of 17-hydroxycorticoids from the adrenal in unstressed dogs, but they did not study the effect of this drug on the response to stress. The present experiments indicate that chlorpromazine does not inhibit ACTH secretion in surgically stressed dogs.

Chronic administration of large doses of this tranquilizer also failed to inhibit the response to the stress of immobilization. There is good evidence that this response is due to psychic, rather than physical factors (Ganong 1961). It is blocked by lesions of the median eminence and dorsal hypothalamus and by destruction of the amygdaloid nuclei (Ganong et al. 1955; Ganong 1961).

A slight increase in adrenal weight and hypertrophy of the zona fasciculata was produced by chlorpromazine. This adrenal hypertrophy probably accounts for the greater than normal ACTH response seen in the chlorpromazine-treated dogs, since the 17-hydroxycorticoid output following a given dose of ACTH is greater when the adrenal cortex is hypertrophic (Ganong & Hume 1956). Reiss (1958) and Talwalker et al. (1960) have reported that chronic administration of chlorpromazine causes adrenocortical hypertrophy in the rat. There is considerable evidence that the drug causes an acute increase in ACTH secretion in the monkey (Harwood & Mason 1957) and the rat (Holzbauer & Vogt 1954; Olling & de Wied 1956; Mahfouz & Ezz 1958; Nasmyth 1955), although others failed to observe this effect in the rat (Sevy et al. 1957).

ARON et al. (1953) first reported that when chlorpromazine was administered to rats before various stresses, the drop in adrenal ascorbic acid normally produced by the stress was blocked. The inhibition of the adrenal ascorbic acid response has generally been confirmed by other investigators (Cheymol et al. 1954; Hamburger 1955; Olling & de Wied 1956; Van Peenen & Way 1957; Sevy et al. 1957; Mahfouz & Ezz 1958), although some authors did not observe this inhibition (Holzbauer & Vogt 1954; Nasmyth 1955). The concensus of opinion, therefore, appears to be that chlorpromazine itself causes adrenal ascorbic acid depletion in rats, but when administered one to four hours before stress, it prevents the usual stress-induced decrease in ascorbic acid. Morphine and reserpine also cause adrenal ascorbic acid depletion in rats, but when administered before stress, they prevent the stress response (Woodbury 1958). Kitay et al. (1959) showed that even epinephrine had this effect. He suggested that anything that acutely stimulated ACTH secretion in the rat could exert a transient »blocking« effect because when the stress was applied after the drug-pituitary ACTH content was depleted. However, it is now known that a lack of adrenal ascorbic acid depletion does not always indicate the absence of an increase in adrenocortical secretion (Ganong & Forsham 1960). Recently
Maickel et al. (1961) have confirmed Kitay's observation that reserpine decreases pituitary ACTH content, and presented other evidence suggesting that Kitay's hypothesis is correct. However, Montanari & Stockham (1962) found that even though there was no adrenal ascorbic acid depletion when a second dose of reserpine was injected in rats, the plasma corticosterone levels rose. It would be interesting to study the effect of pretreatment with chlorpromazine on the plasma corticosterone level or the adrenal venous output of this hormone in stressed rats.

In humans, it has been reported that basal 17-hydroxycorticoid excretion declines during chlorpromazine treatment (Tin et al. 1956; Sulman & Winnik 1956; Kinberger et al. 1956; Fotherby et al. 1959), while others have not observed this depression (Georges & Cahn 1953; Gold et al. 1960). It is worth noting that the experiments in which a decrease in basal adrenal secretion was observed were generally performed on psychotic patients, and the decline could be due to the relief of anxiety and tension by the drug. Inhibition of the adrenocortical response to the stresses of insulin hypoglycaemia (Christy et al. 1957), typhoid vaccine (Fotherby et al. 1959) and methopyrapone (Gold et al. 1960) in humans by chlorpromazine has been reported, but the response to surgical trauma is normal (Vandewater & Gordon 1955; Gold et al. 1960). Christy et al. (1957) claimed that the response of the adrenal cortex to ACTH was normal during chlorpromazine treatment, but the data of Gold et al. (1960) and of Sloane et al. (1958) indicate that the adrenocortical response is decreased. If chlorpromazine does indeed inhibit the responsiveness of the adrenal to ACTH in man, all the reports of decreased responses to stimuli that increase ACTH secretion could be explained on this basis. The present experiments show that such inhibition does not occur with five days of treatment in the dog, but a species difference might well be present.

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REFERENCES


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