Independence of Antimineralocorticoid and Catatoxic Effects of Various Steroids

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Abstract □ Several steroids were assayed by means of Kagawa's test to determine whether the catatoxic or detoxifying effect depends upon antimineralocorticoid potency. In adult male rats, ethylestrenol, norbolethone, progesterone, triamcinolone, prednisolone, and hydroxydione were found free of antimineralocorticoid effects at the dose of 1 mg. Similar results were obtained in young female rats pretreated with as much as 10 mg. of these steroids. Thus, this type of nonspecific resistance-increasing effect of certain steroids appears to be unrelated to antimineralocorticoid potency.

Keyphrases □ Steroids—antimineralocorticoid, catatoxic effects, independence □ Antimineralocorticoid activity—steroids □ Catatoxic activity—steroids □ Atomic absorption spectroscopy—analysis

Recently it was observed in this laboratory that pretreatment with antimineralocorticoid or anabolic steroids diminishes the anesthesia induced by pentobarbital, progesterone, or hydroxydione and prevents the toxicity of digitoxin, dimethylbenz(a)anthracene, indomethacin, and a large variety of unrelated compounds (1–3). This protection is at least partly due to the induction of drug-metabolizing enzymes since spironolactone, norbolethone, or ethylestrenol increases the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent aliphatic hydroxylation of pentobarbital and hexobarbital as well as the *N*-dealkylation of aminopyrine in the hepatic microsomes (4, 5).

The pharmacologic classification of steroid hormones and their derivatives is based upon the fact that some of their actions are separable and independent whereas others are merely inseparable subordinate manifestations of one of these basic activities (1, 6). The stimulation of drug-metabolizing enzymes has already been attributed to different hormonal actions. In studying the role of androgens, Quinn *et al.* (7) observed that the enzymatic degradation of hexobarbital is faster in males than in females. Booth and Gillette (8) found that the activation of drug-metabolizing enzymes by testosterone derivatives more closely parallels their anabolic than their androgenic activity. Regarding the role of female sex hormones, it was found that chronic treatment of ovariectomized rats with progesterone increases the hepatic detoxification of chlorpromazine (9). Remmer (10) noted that glucocorticoids also influence the enzymatic degradation of drugs.

It is of interest whether the catatoxic effect of steroids is dependent on one of their specific hormonal properties. Obviously, not all these steroids share androgenic, anabolic, luteoid, or glucocorticoid activities. However, depending on dosage and under special circumstances, anabolic steroids and progesterone can reveal antimineralocorticoid properties (11). To determine whether the antimineralocorticoid activity is a common function of catatoxic steroids, the authors investigated the effect of norbolethone, ethylestrenol, and progesterone as well as of triamcinolone or prednisolone in Kagawa's test (12), and compared the results with the classic antimineralocorticoid action of spironolactone and SC-11927. The hormonally inactive hydroxydione was used as a control. In a repetition of this experiment on young female rats, the steroids were administered in the same manner and at the same dosage as in the previous detoxification studies (1–5).

MATERIALS AND METHODS

First Experiment—One hundred and forty-four male Sprague-Dawley rats of the Holtzman Farms (Madison, Wis.) with a mean initial body weight of 150 g. (range 140–160 g.) were bilaterally adrenalectomized, under ether anesthesia, by the lumbar route and maintained on sucrose and tap water. Approximately 24 hr. postoperatively the rats were divided and treated as indicated in Table I. Sodium chloride[1] (2.5 ml. of 0.85% solution), desoxycorticosterone acetate (DOC-Ac)[2] (6 or 12 mcg. in 0.2 ml. Mazola corn oil), as well as spironolactone,[3] SC-11927,[3] ethylestrenol,[4] norbolethone,[5] progesterone and prednisolone,[6] triamcinolone,[7] or hydroxydione[8] (1 mg. in 0.5 ml. Mazola corn oil) were administered subcutaneously.

The bladder of the animal was emptied by applying slight pressure before placing four or five of them in a metabolic cage. During the period of urine collection, no food or water was provided. Voiding was again induced at the end of a 4-hr. period for complete recovery of urine. Using the Unicam SP 90 atomic absorption spectrophotometer, pooled specimens were analyzed three times for total sodium and potassium excretion (13); these values were used for the calculation of the log (Na × 10)/K ratio. Blocking activity was measured by reversal of the Na/K effect of DOC, as described by Kagawa (12).

Second Experiment—One hundred and seventeen female Sprague-Dawley rats of the Holtzman Farms with a mean initial body weight of 100 g. (range 90–110 g.) were divided and treated as indicated in Table II immediately after adrenalectomy. Spironolactone, SC-11927, ethylestrenol, norbolethone, progesterone, triamcinolone, prednisolone, or hydroxydione was administered twice daily for 3 days (10 mg. in 1 ml. H_2O *per os*). Groups 13–22 were maintained on Purina Laboratory Chow and 0.85% NaCl as drinking fluid during the first 2 days of this pretreatment; on the 3rd day they received sucrose cubes and tap water. Approximately 72 hr. postoperatively, the pretreatment was followed by the administration of NaCl, DOC, and various steroids as well as by urine collection and electrolyte determinations, as described in the first experiment.

The differences between the Na/K excretion values were statistically evaluated by variance analysis.

RESULTS

The end-point for antimineralocorticoid activity is based on the log urinary Na/K ratio, which may reflect various changes in the in-

[1] Fisher Scientific Co.
[2] Ciba Co. Ltd.
[3] G. D. Searle & Co.
[4] Organon Inc.
[5] Wyeth Laboratories Inc.
[6] Schering Corp.
[7] Lederle Laboratories
[8] Pfizer Ltd.

Table I—The Effect of Various Steroids on Desoxycorticosterone Acetate-Induced Urinary Electrolyte Excretion in Kagawa's Test

Group	Number of Animals	Treatment[a] Steroid, 1 mg.	Treatment[a] DOC-Ac, mcg.	Urinary Excretion[b] μequiv. of Na	Urinary Excretion[b] μequiv. of K	log Na × 10/K Ratio in Urine
1	27	—	—	960	230	1.63
2	9	—	6	770[c]	360[c]	1.33[c]
3	27	—	12	590[c]	400[c]	1.17[c]
4[d]	9	Spironolactone	12	790[e]	320	1.39[e]
5	9	SC-11927	12	760[e]	310	1.39[e]
6	18	Ethylestrenol	12	480[e]	360	1.13
7	9	Norbolethone	12	340[e]	260	1.11
8	9	Progesterone	12	370[e]	270	1.14
9	9	Triamcinolone	12	860[e]	1590[e]	0.73[e]
10	9	Prednisolone	12	910[e]	1790[e]	0.71[e]
11	9	Hydroxydione	12	620	400	1.08

[a] In addition all animals were adrenalectomized 24 hr. earlier and were injected with 2.5 ml. 0.85% NaCl s.c. at the beginning of urine collection. [b] Total electrolyte excretion in sample of nine animals. [c] = $p < 0.05$ as compared with Group 1. [d] Groups 4–7 received the most potent catatoxic steroids. [e] = $p < 0.05$ as compared with Group 3.

dividual cations. Accordingly, Tables I and II list both Na and K means in order that distinctive electrolyte effects of the steroids can be surfaced for interpretation.

First Experiment (Table I)—Compared with the adrenalectomized controls (Group 1), 6 mcg. of DOC-Ac (Group 2) lowered the urinary log (Na × 10)/K ratio and 12 mcg. (Group 3) was even more active ($p < 0.05$ as compared with Group 2). As expected, both spironolactone (Group 4) and SC-11927 (Group 5) reversed the effect of 12 mcg. DOC-Ac. No significant antimineralocorticoid activity was revealed by ethylestrenol (Group 6), norbolethone (Group 7), progesterone (Group 8), or hydroxydione (Group 11). Triamcinolone (Group 9) and prednisolone (Group 10) even further diminnished the urinary Na/K ratio, already decreased by 12 mcg. DOC-Ac (Group 3).

Second Experiment (Table II)—In comparison to the adrenalectomized controls (Group 12), 6 mcg. of DOC-Ac (Group 13) also significantly lowered the urinary log (Na × 10)/K ratio in young female rats, and 12 mcg. (Group 14) was still more active ($p < 0.05$ as compared with Group 13). Spironolactone (Group 15) or SC-11927 (Group 16) counteracted the effect of 12 mcg. DOC-Ac (Group 14). None of the other steroids (Groups 17–22) revealed antimineralocorticoid properties.

DISCUSSION

Kagawa (11) showed that the influence of spironolactone and its derivatives on renal electrolyte excretion is due to a specific antagonism of mineralocorticoid effects. Thus, as expected, spironolactone and SC-11927 reversed the action of 12 mcg. DOC-Ac in both experiments.

It was demonstrated that high doses of testosterone and of many of its derivatives have antimineralocorticoid properties; 18,19-dinortestosterone proved to be active even in small amounts (11). Under the experimental conditions reported here, ethylestrenol or norbolethone failed to show antimineralocorticoid activity, even when a high-dose level was maintained over several days as in the second experiment. Progesterone also proved to possess a DOC-Ac blocking potency similar to that of spironolactone; however, it also resembled DOC-Ac in its effect on electrolyte excretion when given alone and in large doses (14). This complex action and the differences in dosage might explain why progesterone failed to exhibit antimineralocorticoid effects in the experiments. In agreement with expectations, triamcinolone, prednisolone, or hydroxydione was completely devoid of any antimineralocorticoid activity in both experiments. After the administration of triamcinolone or prednisolone, there was a marked decrease in urinary log (Na × 10)/K ratio in male (first experiment) but not in female rats (second experiment). This is probably due to the rats receiving large doses of these steroids for 3 days, thus leading to potassium depletion; therefore, in the actual Kagawa test the last dose of triamcinolone or prednisolone did not so markedly influence potassium excretion.

The lack of correlation between the anabolic, androgenic, luteoid, or glucocorticoid activity of some steroids and their resistance-increasing effect against various compounds was obvious without detailed investigations. The present results demonstrate that even under the experimental conditions used in the detoxification experiments (1–5), the highly active microsomal enzyme inducers, ethylestrenol and norbolethone, do not share the antimineralocorticoid activity of spironolactone and its derivatives. It is noteworthy that spironolactone pretreatment prevents the anesthetic effect of progesterone, DOC-Ac, and hydroxydione, even after bilateral nephrectomy (15). Thus, the catatoxic effect of spironolactone is also manifest under conditions in which its classic antimineralocorticoid property could not play any role.

Table II—The Effect on Kagawa's Test of High Doses of Various Steroids Administered to Young Female Rats

Group	Number of Animals	Treatment[a] Steroid, 10 mg.	Treatment[a] DOC-Ac, mcg.	Urinary Excretion[b] μequiv. of Na	Urinary Excretion[b] μequiv. of K	log Na × 10/K Ratio in Urine
12	18	—	—	1250	450	1.44
13	9	—	6	1010[c]	520	1.29[c]
14	18	—	12	800[c]	630[c]	1.11[c]
15[d]	9	Spironolactone	12	1320[e]	480[e]	1.44[e]
16	9	SC-11927	12	1300[e]	510[e]	1.41[e]
17	18	Ethylestrenol	12	940[e]	700	1.13
18	9	Norbolethone	12	590[e]	480	1.09
19	9	Progesterone	12	630[e]	480	1.12
20	9	Triamcinolone	12	1090[e]	850[e]	1.11
21	9	Prednisolone	12	950[e]	830[e]	1.06
22	9	Hydroxydione	12	750	630	1.08

[a] In addition all animals were adrenalectomized 72 hr. earlier and then treated with steroids (10 mg. twice daily) and given 2.5 ml. 0.85% NaCl s.c. at the beginning of urine collection. [b] Total electrolyte excretion in sample of nine animals. [c] = $p < 0.05$ as compared with Group 12. [d] Groups 15–18 received the most potent catatoxic steroids. [e] = $p < 0.05$ as compared with Group 14.

These results support the view that the catatoxic action of different steroids is not subordinate to any presently known specific hormonal action (1).

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 27, 1969, from the *Institut de Médecine et de Chirurgie Experimentales, Université de Montreal, Montreal, Canada*

Accepted for publication December 16, 1969.

This work was supported by grants from the Ministére de la Santé, Québec, and from the Medical Research Council of Canada (Block Term Grant MT-1829). The authors wish to thank the companies mentioned in the *Materials and Methods* section for their contributions of the drugs used. The technical assistance received from Mrs. C. Jubert and Miss E. Aclément is also gratefully acknowledged.

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Subcutaneous Absorption Kinetics of Benzyl Alcohol II

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Abstract □ Under multiple-dosing conditions at a subcutaneous site, equations were derived which permit one to estimate the number of doses, n, required to approach within ± 1% (or any other fixed fraction) of the asymptotic minimum level: $n \geq 3.3219 [(t_{0.5})/\tau] \log_{10} Q$, where $Q > +1$. Here $t_{0.5}$ is the absorption half-life of benzyl alcohol from a subcutaneous absorption cell, τ is the dosing interval, Q (always positive) equals $(\beta - 1)/(\alpha - 1)$, α equals $B^{(n)}_{\text{min.}}/B^{\infty}_{\text{min.}}$ (and is 0.99 or 1.01 in this example), and β equals $B''/B^{\infty}_{\text{min.}}$, where B'' equals $Bi - Bm$. Definitions: $B^{(n)}_{\text{min.}}$ = amount of benzyl alcohol in the cell per unit area of subcutaneous tissue one τ after the nth dose, $B^{\infty}_{\text{min.}}$ = asymptotic minimum amount of benzyl alcohol in the cell per unit area, Bi = initial dose of benzyl alcohol per unit area, and Bm = constant maintenance dose of benzyl alcohol per unit area ($Bi \geq Bm$). The benzyl alcohol disappears from the cell in an apparent monoexponential manner.

Keyphrases □ Benzyl alcohol—subcutaneous absorption □ Kinetics—benzyl alcohol, subcutaneous absorption □ Equations—dosage numbers/subcutaneous area for asymptotic minimum level

In a recent report from this laboratory (1), a multiple-dosing procedure was used in connection with a study of the subcutaneous (s.c.) absorption kinetics of benzyl alcohol (BA) dissolved in normal saline (NS). The BA in NS solution was contained in a glass absorption cell affixed to the moist s.c. tissue of an anesthetized rat by a silicone adhesive. The solution was kept homogeneous by means of a vibrating stirrer. The use of this cell made it possible to control the area of s.c. tissue exposed to the drug solution at all times and to sample the cell's contents periodically.

Although the number of doses per unit area (p.u.a.) of s.c. tissue needed to approach within ±1% of the asymptotic minimum value were shown in Table IV of the previous report (1), details of the mathematical calculations were omitted. The purpose of this note is to derive the equations needed to make this estimation.

THEORETICAL

Under multiple-dosing conditions, where the mean volume of drug solution in the cell was nearly constant throughout the experiment, and the drug was administered at the times zero, τ, 2τ, 3τ, ..., the following equation can be derived (1):

$$Bc = B' \frac{(1 - e^{-Pn\tau})}{(1 - e^{-P\tau})} \qquad \text{(Eq. 1)}$$

where Bc in this case is the amount of drug in the cell p.u.a. just after the administration of the nth dose p.u.a. In Eq. 1 it is assumed that the drug disappears from the cell in an apparent monoexponential manner and the initial dose p.u.a., Bi, equals the constant maintenance dose(s) p.u.a., Bm, in magnitude. Thus

$$B' = Bi = Bm \qquad \text{(Eq. 2)}$$

The term P in Eq. 1 is the mean penetration coefficient having the units of time^{-1}, n is the integer number of initial and maintenance doses administered, and τ is the constant dosing time interval.

When Bi is larger than Bm, then

$$Bi = B'' + Bm \qquad \text{(Eq. 3)}$$

where B'' is the amount of drug p.u.a. in the cell administered along with Bm as a part of the initial dose p.u.a. and $Bi \geq Bm$. The amount