significantly inhibit DBH activity, in contrast to the results of Kuzuya and Nagatsu [6]. Of interest here is the fact that 0·05% Triton X-100 is still sufficient to measure the total DBH activity.

In conclusion, it appears that ATP in itself exerts no direct stimulation on DBH. However some ATP preparations which are contaminated by copper may artificially increase DBH activity by reversing the effects of endogenous inhibitors. As DBH activity is generally measured with or without ATP the present results provide a good explanation for the considerable discrepancies which exist between numerous reports as regards the copper concentration required to produce maximal DBH activity.

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Influence of adrenocorticotropic hormone, somatotrophic hormone and pregnenolone-16α-carbonitrile on drug response and metabolism

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In intact unlike in adrenalectomized rats, pretreatment with adrenocorticotropic hormone (ACTH) reduces the effects of certain drugs [1]. This action can be only partially simulated by corticosterone, suggesting that some steroids are involved in the effect of ACTH upon drug responses [1].

Somatotrophic or growth hormone (STH) is another agent that influences drug responses [2, 3]. Given as a pretreatment with ACTH, it abolishes the latter's protective effect upon acrylonitrile-induced adrenal apoplexy and mortality [4]. In rats, conjoint treatment with STH and pregnenolone-16α-carbonitrile (PCN) counteracts the beneficial action of the steroid against digitoxin or indomethacin intoxication [5, 6].

PCN, a synthetic nonhormonal catatoxic steroid, possesses no other known effect besides its ability to reduce the toxicity of a large number of drugs [2]. In most cases, its actions are mediated through the induction of drug-metabolizing enzymes in hepatic microsomes [7–10], but it also stimulates certain extramicrosomal enzymes, for example, phosphoprotein phosphatase [11]. Recently, it was found that the reduction of zoxazolamine paralysis time by PCN, unlike that caused by ACTH, is associated with decreased drug concentrations in plasma [12]. This protective effect of ACTH is shared by triamcinolone and corticosterone in that the diminution of zoxazolamine paralysis is not accompanied by lower plasma levels of the toxicant [12, 13]. Distribution studies [14, 15] have not explained the reduction of zoxazolamine paralysis by ACTH and corticoids. Hence, it seemed of value to compare the effects of these agents on drug metabolism. Preliminary reports on this subject have appeared elsewhere [16, 17].

Female Charles River CD® rats (Canadian Breeding Farms & Laboratories Ltd., St. Constant, Que.), with an initial average body weight of 100 g, were maintained *ad lib.* on Purina Laboratory Chow (J. Mondu Inc., Montreal, Que.) and tap water. Unless otherwise stated, each group consisted of 10–15 rats. Every experiment was repeated two to three times.

In the first experiment, PCN (3β-hydroxy-20-oxo-5-pregnen-16α-carbonitrile (Upjohn) was given p.o. (by stomach tube) at a dose level of 1 mg in 1 ml water (as a microcrystal suspension after addition of a trace of polysorbate or Tween 80) twice daily on the first, second and third day and once on the fourth day, 1 hr before zoxazolamine (K. & K. Laboratories) in 1 ml distilled water (homogenized with a trace of polysorbate or Tween 80). The wet adrenal weights were determined on day 5.

In the second experiment, 5 I.U. of depot ACTH was injected s.c. 24 hr before zoxazolamine (given as described in the first experiment). Bovine STH (Upjohn) was administered once s.c., at a dose level of 2 mg or 5 U (in 0·2 ml distilled water) 28 and 4 hr before the zoxazolamine injection.

In the third experiment, 0·1 mg PCN (a dose just sufficient to diminish zoxazolamine paralysis) was given p.o. twice daily on days 1, 2 and 3 and once on the day 4, 1 hr before zoxazolamine (administered as in the first experiment). STH was injected s.c., always 1 hr before PCN, in doses that were identical to those used in the second experiment.

In the fourth experiment, 1 mg PCN was given twice daily p.o. for 3 days. Depot ACTH (5 I.U.) was injected once s.c. 24 hr before the rats were decapitated (on day 4, approximately 17 hr after the last PCN gavage).

The livers were immediately excised, weighed and washed in an ice-cold aqueous solution of 1·15% KCl, containing 0·02 M tromethamine Tris–HCl buffer, pH 7·4. All the hepatic tissues were subsequently processed at 0–4°,

Table 1. Effect of ACTH, PCN and STH on zoxazolamine paralysis

Group	Pretreatment*	Paralysis time (min)	P value†
1	None (control)	188 ± 10	
2	ACTH	89 ± 11	<0·005
3	PCN (1 mg)	70 ± 9	<0·005
4	STH (twice)	184 ± 12	NS
5	PCN + ACTH	45 ± 6	<0·005‡,§
6	STH + ACTH	177 ± 17	NS‡
7	PCN (0·1 mg)	155 ± 12	<0·05
8	STH (daily)	215 ± 15	NS
9	STH + PCN	238 ± 16	NS‖

* In addition, the rats of all groups were given 10 mg/100 g of body weight of zoxazolamine, i.p.
† P values compared to control. NS = not significant.
‡ P < 0·005 compared to group 2.
§ P < 0·05 compared to group 3.
‖ P < 0·005 compared to group 7.

mostly according to the method of Conney *et al.* [18]. Corresponding liver lobes were homogenized in a Potter–Elvehjem homogenizer (for approximately 1 min, with 8–10 downward strokes of the pestle), using 3 ml of the above-mentioned solution per g of liver. After centrifugation at 9000 *g* for 20 min, the supernatant fraction was transferred into an incubation medium (3 ml) which contained, in final concentration, 5 mM $MgCl_2$, 5 mM glucose 6-phosphate, 0·4 mM NADP (dissolved originally in tromethamine–HCl buffer, pH 7·4) and 0·4 μM of an acidified, aqueous solution of zoxazolamine. A Dubnoff shaking incubator was used for incubation at 37° for 30 min (120 c/min). The reaction was terminated by adding 1 ml of 1 N NaOH. The amount of unchanged zoxazolamine (after extraction into ethylene dichloride, in the presence of sodium borate, and later into hydrochloric acid) was estimated according to the method of Burns *et al.* [19] with a UNICAM SP 8000 spectrophotometer. The proteins were measured in the 9000 *g* supernatant fraction by the method of Lowry *et al.* [20] using bovine albumin as a standard.

In the fifth experiment, 0·1 mg PCN was administered twice daily p.o. for 3 days. STH was given s.c. at a dose level of 2 mg (5 U), always 1 hr before the steroid. The rats were killed by decapitation 17 hr after the last PCN gavage. Zoxazolamine metabolism was assessed as in the fourth experiment.

The results of the first three experiments are presented in Table 1. It can be seen that PCN at the 1 mg dose level and ACTH markedly shortened zoxazolamine paralysis time, whereas PCN at 0·1 mg slightly shortened it. Conjoint ACTH and PCN administration was more significant in this respect than treatment with ACTH or PCN alone. STH by itself or in combination with ACTH or PCN had no significant effect.

The weight of the adrenal gland in controls (28·5 ± 1·9 mg/100 g of body weight) and in animals treated with PCN (27·1 ± 1·1) was similar. On the other hand, there was significant adrenal enlargement in rats given ACTH (35·5 ± 1·8) or ACTH + PCN (37·4 ± 2·1).

The results of the fourth and fifth experiments are shown in Table 2. PCN, even at the low dose level of 0·1 mg, significantly enhanced the metabolism of zoxazolamine in the 9000 *g* supernatant fraction of the liver, whereas ACTH exerted no pronounced action. Conjoint administration of PCN and ACTH also accelerated the biotransformation of zoxazolamine, but this increase was not significantly greater than that in animals given PCN alone. STH given alone did not significantly alter zoxazolamine metabolism and PCN had a stimulatory effect even in combination with STH.

Liver weight was significantly augmented by both PCN (1 mg) and ACTH. The results were more pronounced in rats treated with PCN + ACTH than in those which received PCN alone. The amount of hepatic protein was significantly increased only by STH.

The results presented here demonstrate that pretreatment with either ACTH or PCN reduces zoxazolamine paralysis time. This effect of ACTH and PCN seems to be synergistic, but the latter does not modify the adrenotrophic action of the former.

PCN, unlike ACTH, considerably enhances the metabolism of zoxazolamine by the 9000 *g* supernatant fraction of the liver, a finding which is in agreement with earlier reports; the steroid accelerates the oxidation of several drugs (e.g. pentobarbital, aniline) [7, 8, 10], while ACTH is virtually inactive in this respect [21]. On the other hand, despite the synergism between ACTH and PCN in diminishing zoxazolamine paralysis, conjoint administration of the two does not markedly alter the stimulating effect of PCN on drug metabolism. Other experiments (not reported here) have shown that the increase in liver weight caused by ACTH, and especially by ACTH + PCN, is due to hepatic deposition of lipids and glycogen. The hepatic microsomal enzyme inhibitor, SKF 525-A, blocks the effect of PCN, but not that of ACTH, upon zoxazolamine paralysis [15].

STH does not influence the duration of zoxazolamine paralysis, but abolishes the protective actions of both ACTH and PCN. However, it does not markedly alter the stimulating effect of PCN upon drug metabolism. Fur-

Table 2. Influence of PCN, ACTH and STH on the metabolism of zoxazolamine by the 9000 *g* supernatant fraction of the liver

Group	Treatment	No. of rats	Zoxazolamine metabolism (μmoles/g liver/hr)	P value*	(%)	Proteins (mg/g liver)	P value*	Liver wt (g/100 g body wt)	P value*
1	None (control)	8	2·18 ± 0·12		100	93·42 ± 0·66		4·70 ± 0·16	
2	PCN (1 mg)	7	4·77 ± 0·17	<0·005	218	93·36 ± 1·41	NS	5·21 ± 0·08	<0·05
3	ACTH	7	2·47 ± 0·18	NS	113	90·76 ± 1·76	NS	5·41 ± 0·08	<0·01
4	PCN + ACTH	8	5·19 ± 0·16	<0·005†	237	90·46 ± 0·97	NS	6·22 ± 0·08	<0·005‡
5	PCN (0·1 mg)	7	2·90 ± 0·22	<0·05	133	92·16 ± 1·89	NS	4·94 ± 0·06	NS
6	STH	7	1·79 ± 0·16	NS	82	105·52 ± 1·56	<0·05	4·53 ± 0·08	NS
7	PCN + STH	7	2·56 ± 0·12	<0·05§	117	94·92 ± 2·61	NS	4·84 ± 0·19	NS

* P values compared to control. NS = not significant.
† P is NS compared to group 2.
‡ P < 0·005 compared to group 2.
§ P is NS compared to group 5.

thermore, STH interferes with the reduction of zoxazolamine paralysis by ACTH, which is not mediated through enhanced metabolism of the toxicant. These facts suggest that growth hormone alters the effects of PCN, ACTH and/or zoxazolamine at the periphery and not at the level of hepatic drug-metabolizing enzyme activity. On the other hand, STH represses the induction of hepatic tyrosine-α-ketoglutarate transaminase by glucocorticoids [22]. Phenobarbital, given 24 hr after a mixture of STH + ACTH + prolactin, still significantly increases the metabolism of aminopyrine, hexobarbital and ethylmorphine in male rats [23], although the content and rate of reduction of hepatic microsomal cytochrome P-450 as well as the liver metabolism of hexobarbital and aniline are lowered by STH [24]. A mutual antagonism exists between STH and corticoids: the latter inhibit the body weight gain and the stimulation of bone growth elicited by STH [25].

The effects of ACTH, glucocorticoids and STH upon drug responses do not appear to be identical to those of stress, which depend mostly on the stressor, time of exposure, strain of the animal, etc. [26, 27]. Acute stress usually induces drug-metabolizing enzymes in the liver and diminishes drug levels in blood and target organs [28–30]. Stressors can prolong the effects of drugs, decrease their biotransformation, and impair the activity of sex-dependent but not of sex-independent enzymes in hepatic microsomes [31, 32]. Thus, the influence of stress might be direct and specific to the stressor, while that of ACTH, STH and other hormones could be direct or mediated. Yet, perhaps all these direct and indirect actions of stress—manifested through ACTH, STH or groups of other hormones—merely constitute a reaction scale, where each of the following effects may have its own significance: (1) the rapid increase of drug biotransformation after exposure to stress; (2) the interference by STH with the actions of certain drugs; and (3) as we recently demonstrated with the reduction of zoxazolamine paralysis by ACTH [33], the acceleration of substrate excretion into the bile and urine and the ACTH-induced diminution of free drug levels through augmentation of protein-bound zoxazolamine.

In summary, pretreatment with ACTH or PCN diminishes zoxazolamine paralysis, but only the steroid enhances the metabolism of the drug by the 9000 *g* supernatant fraction of the liver. There seems to be a synergism between ACTH and PCN in reducing zoxazolamine paralysis but not in stimulating substrate biotransformation. This indicates that ACTH and PCN have qualitatively different mechanisms of action. STH does not markedly alter zoxazolamine paralysis or metabolism but counteracts the protective effects of ACTH and PCN.

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