Effect of various steroids and ACTH on methyprylon plasma levels and sleeping time in rats

Methyprylon, a popular sedative, is mildly hypnotic in therapeutic doses. Little is known about its distribution and metabolism beyond the fact that, in dogs and man, less than 3% of an orally-administered dose appears unchanged in the urine, and similar amounts of 3,3-diethyl-2,4-dioxo-5-methyltetrahydropyridine are excreted through the same route. In rats given methyprylon, about 10% is eliminated unchanged and 10% in the form of the above-mentioned metabolite (Sharpless, 1971; Randall, Iliev & Brandman, 1956).

Certain pharmacologic agents, including steroids, reduce the biological half-life of many drugs, thereby decreasing the duration and intensity of their actions (Conney, 1967; Selye, 1971a). It has been established that steroidal compounds play a decisive role in determining the resistance of the body against the most varied types of injury (Selye, 1971b). Based on these properties, the steroids have been classified according to their mechanism of action into: steroids that improve host-tissue tolerance by permitting coexistence with the pathogen (e.g., by suppressing nonspecific inflammatory or allergic reactions against it), and steroids that enhance the detoxication of endogenous and exogenous toxicants via induction, activation, decreased degradation of drug-metabolizing enzymes and/or accelerated substrate elimination. We have shown in a study on these mechanisms (Kourounakis, Szabo & others, 1973) that the plasma concentrations of drugs are characterized by the following relations:

For the first group of steroids $C_{cd} \simeq C_1$ and $C_{cd} > C_2$, and for the second group $C_{cd} < C_1$ and $C_{cd} \simeq C_2$ where C_{cd} = plasma concentration of the drug in pretreated animals at the termination of the pharmacologic response; C_1 = blood level of the drug in the not yet recovered controls, killed when the pharmacologic response has disappeared only in the pretreated group; C_2 = plasma concentration in the recovered controls killed at the termination of the pharmacologic response.

Based on these criteria, we undertook to investigate the correlation between the *in vivo* effects of steroids, as well as ACTH, and the plasma concentration of methyprylon. We also wished to clarify whether the protective compounds act through one or the other mechanism of action.

Female Sprague-Dawley rats (Canadian Breeding Farm & Laboratories Ltd., St. Constant, Quebec), averaging 100 g (range 90–110 g) and maintained on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water *ad libitum*, were divided into three groups of which the first and second were given 1 ml of water, orally, twice daily for 3 days and once on the fourth day. The rats of the third group received pregnenolone-16α-carbonitrile [PCN (Upjohn)], spironolactone (Searle), triamcinolone (Squibb), 9α-fluorocortisol acetate (Upjohn), corticosterone (Merck, Sharp & Dohme), or desoxycorticosterone acetate [Doca (Upjohn)] at a dose of 1 mg in 1 ml water (homogenized with a trace of Tween 80), orally, also twice daily for 3 days and once (1 h before methyprylon) on the fourth day. ACTH [Synacthen Depot (Ciba)] was administered at the 5 IU (50 μg, s.c.) dose level, once on the third day. Methyprylon [13 mg per 100 g, s.c. in 0·2 ml water (Hoffmann-La Roche)] was given to all groups on the fourth day.

Blood samples were taken from the non-recovered controls and pretreated animals when the latter regained the righting reflex, and from the recovered controls when their righting reflex reappeared. These samples were analysed according to Randall, Iliev & Brandman (1956). Standards and blanks were prepared with drug-free plasma from pretreated and un-pretreated animals.

The methyprylon concentration in the blood of rats given PCN or spironolactone was significantly lower than that of the non-recovered controls (killed when the pre-

Table 1. *Effect of various steroids and ACTH on methyprylon plasma levels and sleeping time.*

	Concentration of methyprylon in plasma (μg ml^{-1})			Sleeping time (minutes)	
Conditioner	Pretreated[a] animals	Nonrecovered[b] controls	Recovered[c] controls	Recovered controls	Pretreated animals
Pregnenolone-16 α-carbonitrile	97·3 ± 7·0 (9)	144·5 ± 5·2*** (10)	111·0 ± 5·2 NS (8)	100·3 ± 15 (8)	14·2 ± 5·7 (10)
Spironolactone	80·2 ± 2·3 (8)	136·4 ± 12·7*** (8)	98·2 ± 7·7* (6)	222·0 ± 20 (6)	82·0 ± 6** (8)
Triamcinolone	131·2 ± 10·6 (7)	119·5 ± 8·7 NS (7)	121·0 ± 6·0 NS (6)	101·0 ± 12 (10)	68·0 ± 4*** (10)
Fluorocortisol acetate	102·5 ± 6·3 (8)	122·7 ± 12·7 NS (7)	115·8 ± 12·1 NS (5)	96·3 ± 4·9 (6)	75·6 ± 6·2* (8)
Corticosterone	96·7 ± 3·4 (8)	103·7 ± 5·5 (8)	99·7 ± 3·5 NS (6)	145·7 ± 5·2 (6)	113·4 ± 5·7 NS (8)
Desoxycorticosterone acetate	113·4 ± 10·9 (8)	114·3 ± 5·8 NS (8)	96·2 ± 4·5 NS (7)	146·5 ± 3·5 (6)	110·5 ± 11·1 (8)
ACTH	101·1 ± 7·2 (7)	98·6 ± 4·2 NS (8)	95·0 ± 12·5 NS (5)	101·0 ± 12 (10)	66·0 ± 3 (8)

[a] Killed when the righting reflex was regained.
[b] Killed when the pretreated group regained the righting reflex.
The plasma levels of methyprylon in the recovered and non-recovered controls are compared with the plasma concentration of the drug in the pretreated rats: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$; NS = not significant.
Figures in brackets indicate number of animals.

treated animals regained the righting reflex); it corresponded to the level in recovered controls when the righting reflex reappeared in these rats (Table 1). Thus, PCN and spironolactone fulfilled the relation $C_{cd} < C_1$, $C_{cd} \simeq C_2$. These results are in agreement with recent findings that, among many steroids, PCN—though devoid of any hormonal activity—exerts the greatest prophylactic effect against numerous toxicants *in vivo* (Selye, 1971a, b). This steroid also diminishes zoxazolamine plasma concentrations and paralysis time (Kourounakis & others, 1973). Spironolactone (Aldactone) inhibits the anaesthetic and sedative actions of various compounds (Selye, Mécs & Savoie, 1969; Selye, 1970).

The significant reduction of methyprylon sleeping time in animals pretreated with these two steroids was associated with a fall in the plasma level of the drug, which seems to be due to hepatic microsomal enzyme induction. This hypothesis is supported by the ability of PCN to induce NADPH-cytochrome C-reductase and cytochrome P_{450} (Solymoss, Werringloer & Toth, 1971), as well as by the fact that spironolactone enhances the hydroxylation of many compounds (Solymoss, Classen & Varga, 1969; Solymoss, Varga & Classen, 1970).

Methyprylon sleeping time was slightly decreased in fluorocortisol acetate-treated rats, but the changes in the plasma concentration of the drug were not significant although they displayed a prophylactic pattern characteristic of the second type of mechanism. Both triamcinolone and ACTH significantly inhibited the action of methyprylon and, here, the increase in the blood level was not marked. Judging from the plasma concentration of the drug, these two compounds seemed to exhibit a mechanism of the first kind. In our earlier study (Kourounakis & others, 1973), zoxazolamine paralysis was likewise reduced by triamcinolone and ACTH. Doca significantly shortened methyprylon sleeping time. The changes in the plasma level were minimal, which may reflect a mechanism of the first kind due to decreased receptor sensitivity, altered drug distribution in the body, or interactions at receptor sites. Corticosterone had no effect on methyprylon blood clearance or sleeping time.

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Dithiocarbamate central effects in relation to their possible influence on drug metabolizing enzymes

We have previously shown that administration of diethyldithiocarbamate to rats causes behavioural changes (Mayer & Ebyl, 1971). We now report the effects of two other dithiocarbamate derivatives, dimethyldithiocarbamate and disulfiram, upon the exploratory activity of rats, and also the effect of diethyldithiocarbamate upon drug metabolism as indicated by the disappearance of pentobarbitone from plasma. Spironolactone was used to induce the drug metabolizing enzymes.

The exploratory activity of female albino rats (Wistar, 150–200g) was tested. A plastic box (37 × 21 × 25 cm) equipped with an automatic register for measuring the frequency of standing-up reactions, and having the floor divided into 8 painted equal squares was used. The number of squares crossed by a rat (by at least one half of its body) was counted by a trained observer. No significant difference was found between the data so obtained and those obtained automatically with apparatus described by Mayer & Eybl (1971). The stereotyped movements of the rats perceptible after the amphetamine pretreatment and defined as continuously sniffing, licking and head-moving (the amphetamine stereotypes) were registered at the same time. The sleeping time of male mice (Lysolaje strain) was registered in minutes as the period of lost righting reflex, and calculated as the geometric mean value. Plasma concentrations of pentobarbitone in male mice were estimated according to Tietz (1970).

Sodium diethyldithiocarbamate (DDC, Lachema Brno), sodium dimethyldithiocarbamate (DMDC, Lachema Brno), pentobarbitone (Spofa), and commercial solutions of amphetamine sulphate (Psychoton) were administered intraperitoneally