Biochemical Pharmacology, Vol. 38, No. 12, pp. 2045–2046, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Effect of the acetylcholinesterase inhibitor, soman, on plasma levels of β-endorphin and adrenocorticotropic hormone (ACTH)

(*Received* 27 *May* 1988; *accepted* 16 *December* 1988)

Soman (pinacolyl methylphosphonofluoridate) is a potent irreversible acetylcholinesterase inhibitor. Acute intoxication with this agent is associated with a large increase of adrenal corticosteroids in the blood [1]. Althouth these increases due to soman and other organophosphorus agents [2–4] are assumed to be a result of adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary, the soman-induced rise in corticosterone levels in the mouse is not associated with increased levels of ACTH [5].

Soman toxicity in mice is also associated with a long-lasting antinoceptice effect [6]. Naloxone antagonizes this analgesic response which suggests the involvement of endogenous opioids. Although a significant decrease in β-endorphin levels in the pituitary 24 hr after acute soman poisoning in rats has been reported, there are no differences in the β-endorphin levels in the pituitary of mice at 24 hr after acute soman [7]. The purpose of this study was to measure the corticosterone, ACTH, and β-endorphin levels in plasma of rats after soman and to compare these levels with those caused by immobilization stress which also causes increased levels of these peptides [8].

Methods

Male Sprague–Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN). All rats had free access to food and water and weighed between 290 and 350 g at the time of experimentation. Soman was obtained from the U.S. Army Medical Research Command, Aberdeen Proving Ground, MD.

Rats were given either saline or soman, 80 μg/kg, by s.c. injection in the dorsal part of the neck and were killed 18 min (time interval for development of toxic signs for most rats) later by decapitation. This dose, which approximates the s.c. LD_{50} dose (75 μg/kg), was used because in previous studies it consistently caused seizures after 30 min for all the rats. This dose also depresses the hypothalamic acetylcholinesterase levels to 10–20% of controls after 15 min [9]. For comparative purposes, one group of saline-treated rats was subjected to 18 min of immobilization stress. Rats were immobilized by placing them in polyfilm restraining cones (The Ealing Corp., South Natick, MA). The nose of the rat was placed in the small end of the cone which is open to allow the animal to breathe. The large end of the cone was compressed around the rat's tail and secured by tape. Holes were previously punched into the cone to allow ventilation for the trunk portion of the rat's body.

Peak signs of intoxication for each rat were rated prior to decapitation according to the following scale: 0 = asymptomatic, 1 = chewing behavior, 2 = above plus salivation, 3 = above plus muscle fasciculations and tremors, 4 = above plus clonic seizures, and 5 = above plus tonic seizures.

Plasma samples were obtained from trunk blood and were stored at −70° prior to the assays for corticosterone, ACTH, and β-endorphin. Plasma levels of β-endorphin and ACTH were determined by radioimmunoassays (Immuno Nuclear Corp., Stillwater, MN). Cross-reactivity of the ACTH antibody is less than 0.01% with α-melanocyte stimulating hormone, β-endorphin, and β-lipotropin. Cross-reactivity of the β-endorphin antibody is less than 5% with β-lipotropin and less than 0.01% with dynorphin, methionine enkephalin, and ACTH. The corticosterone plasma levels were measured by the Corticosterone Radioimmunoassay (Radioassay Systems Laboratory, Inc., Carson, CA).

The data were analyzed by one-way analysis of variance, and the significance of differences between means was determined by utilizing the Newman–Keuls range test.

Results and discussion

The results in Table 1 show that rats administered soman had a range of toxic signs (excessive grooming, salivation, seizures) with an average toxic sign-score of 3.1 out of a possible 5. The corticosterone, β-endorphin, and ACTH levels in both soman-treated and stressed groups were significantly different ($P < 0.05$) from the control group (saline-treated, nonstressed). The ACTH levels of the soman-treated rats were also significantly higher than the stressed group.

It has been reported that ACTH levels are not elevated 3 hr after toxic doses of soman in mice, despite large increases in corticosterone levels [5]. It was proposed that soman may have a direct effect on the adrenal cortex or that there may be a decreased metabolism of corticosterone by the liver due to soman-induced hypothermia. The results of the present study indicate that the large increase in corticosterone levels is associated with increases in ACTH levels. In addition to species differences, the discrepancy between these studies may be due to the time intervals for sampling after dosing (18 min vs 3 hr). In another study, in which rats were subjected to prolonged immobilization stress, the initial increased plasma ACTH levels declined rapidly after 30 min while the corticosterone levels remained elevated for the duration of stress [10]. It is apparent that ACTH does play a role in the soman-induced high corticosterone levels, at least in the early time intervals after toxic doses in the rat.

The increased levels of corticosterone and β-endorphin in stressed rats appeared to be of the same magnitude as those observed after soman. Stress causes the release of corticotropin releasing hormone from the hypothalamus which, in turn, causes the release of ACTH and β-endorphin from the anterior pituitary [8, 11]. Both ACTH and β-endorphin are derived from the same precursor peptide [12]. Therefore, stress probably plays a role in the endocrine response to soman. The levels of ACTH after soman were significantly higher than those observed in the stressed rats and may indicate an additional release of this peptide due to soman. A direct central effect of soman, through its inhibition of acetylcholinesterase in the hypothalamus, may contribute to the increased levels of ACTH. Acetylcholine and its agonists stimulate the release of corticotropin releasing hormone from the hypothalamus both *in vitro* and *in vivo* [13, 14]. However, the slight rise in β-endorphin levels in soman-treated rats, when compared to non-treated stressed rats, was not statistically significant.

In summary, acute soman toxicity caused increased plasma levels of corticosterone, ACTH, and β-endorphin. The magnitude of these increases was similar to those observed in rats subjected to immobilization stress except for the ACTH levels which were significantly higher after soman. The high corticosterone levels observed after soman appear to be due to increased ACTH levels, at least in the early time intervals after soman dosing.

Table 1. Effect of an acute soman dose on the plasma levels of corticosterone, ACTH and β-endorphin*

Treatment	Average toxic sign score	Corticosterone (ng/ml)	ACTH (pg/ml)	β-Endorphin (pmol/L)
Saline, non-stressed	—	111 ± 29	85 ± 5	27 ± 7
Soman, 80 μg/kg	3.1	410 ± 21†	586 ± 105‡	115 ± 7†
Saline, stress	—	489 ± 20†	379 ± 45†	108 ± 5†

* Blood sample was obtained 18 min post-treatment. Values are means ± SE, N = 8.
† Significantly different ($P < 0.05$) from saline-nonstressed group.
‡ Significantly different ($P < 0.05$) from both groups.

Acknowledgements—This investigation was supported by Contract DAMD 17-83-C-3193 from the United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

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Biochemical Pharmacology, Vol. 38, No. 12, pp. 2046–2049, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Differential effects of cimetidine, ranitidine and famotidine on the hepatic metabolism of estrogen and testosterone in male rats

(*Received* 19 *September* 1988; *accepted* 13 *December* 1988)

Cimetidine (*N*″-cyano-*N*-methyl-*N*′-[2[[(5-methyl-1*H*-imidizol-4-yl)methyl]thio]-ethyl]-guanidine) is a histamine H_2-receptor antagonist which contains an imadazole ring and binds, as a type II ligand, to cytochrome P-450 [1–5]. Such interactions lead to inhibition of cytochrome P-450 function and decreased metabolism of exogenous pharmacological