Increased Plasma Corticosterone and Decreased Plasma Thyroid-stimulating Hormone Levels in Rats Treated with Vincristine

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SUMMARY

A single i.p. injection of vincristine (1000 µg/kg) into rats increased the plasma concentration of corticosterone after a latent period of 4 hr; the effect lasted for 48 hr. The response was dose related with the threshold dose being 100 µg/kg and the maximal effect occurring after 250 µg/kg. Vincristine also increased plasma corticosterone levels in hypophysectomized rats, suggesting that the drug may have a direct action on the adrenal gland. The injection of 500 or 1000 µg/kg also reduced the plasma concentration of thyroid-stimulating hormone. Twelve and 18 hr after the injection of vincristine (1000 µg/kg), the plasma concentration of thyroid-stimulating hormone was reduced, whereas the hypothalamic content of norepinephrine, a neurotransmitter involved in the regulation of thyrotropin-releasing hormone-stimulating hormone secretion, remained unchanged.

The adrenocortical stimulation produced by vincristine may play some role in the antineoplastic effects of this drug.

INTRODUCTION

In addition to their antitumor actions, some antineoplastic drugs can produce changes in endocrine systems. For example, busulfan can produce selective pituitary insufficiencies in humans (25), cyclophosphamide can induce superovulation in rats (17), and methotrexate can reduce some endocrine actions of vincristine, an antimitotic agent (12). These changes are of interest because alterations in endocrine states may reinforce or impair the cytotoxic effects of the antineoplastic compound.

Since cytoplasmic microtubules have been implicated in hormonal secretion processes (13), we have investigated some endocrine actions of vincristine, an antimitotic agent that binds to protein tubulin, an important component of microtubules. Specifically, we examined the effects of vincristine on the plasma concentrations of corticosterone and TSH in rats. In addition, since microtubules and their protein subunits are cellular components of fast axonal transport systems and neurosecretion-neurotransmission processes (23), we examined the effects of vincristine on the hypothalamic content of norepinephrine, a neurotransmitter involved in regulating the secretion of inhibiting or releasing hormones (1, 18, 19).

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 200 to 250 g were housed 4/cage in a room with automatic light control that provided light (5 a.m. to 7 p.m.) and 10 hr dark each day. Food (commercial rat chow) and water were available ad libitum. The animal room was maintained at a constant temperature of 25 ± 1° for at least 10 days before the experiments. Animals were killed by decapitation. Trunk blood was collected and centrifuged, and plasma was promptly frozen. In some experiments, brains were removed, and hypothalami were quickly dissected according to the method of Weiner and Ganong (27). Three hypothalami were pooled, weighed, and homogenized in 5% trichloroacetic acid and frozen for subsequent analysis of norepinephrine.

Hypophysectomy was performed by parapharyngeal approach 20 to 23 days before the experiment. At autopsy, completeness of hypophysectomy was judged by inspection of the pituitary site under a dissecting microscope.

Experiment A. Vincristine (Eli Lilly and Co., Indianapolis, Ind.) was administered i.p. at the dose of 1000 µg/kg; groups of animals were killed 1, 4, 8, 18, 36, 48, and 60 hr after administration. Control rats received the drug solvent (Eli Lilly and Co.) in the same volume as the treated animals. All the experiments in which plasma corticosterone was assayed were programmed in order to sacrifice the animals at 8 a.m., when plasma corticosterone is at the lowest levels.

For studying the relationship between the administered dose and the adrenocortical activation, various doses of vincristine were injected i.p. 18 hr before the sacrifice.

Experiment B. Both normal and hypophysectomized rats received vincristine (250 µg/kg i.p.), drug solvent (in the same volume as the treated animals i.p.) 18 hr before the sacrifice. ACTH (100 milliunits/kg i.p.) was injected 12 min before the sacrifice since, in male rats, this appears to be the optimal time for detecting the highest concentration of corticosterone in the plasma (6).

Experiment C. The animals were treated with vincristine (1000 µg/kg i.p.) 6, 12, and 18 hr before the sacrifice. Plasma TSH levels were determined by radioimmunoassay. Hypothalamic norepinephrine was also measured. All the animals were killed at 11 a.m., the time at which plasma...
TSH reaches the highest levels of circadian variation in male rats (14).

To study the dose-effect relationship on TSH secretion the Vinca alkaloid was administered at doses of 50, 100, 250, 500, and 1000 µg/kg 18 hr before killing.

**Analytical Procedures.** Plasma corticosterone was estimated fluorometrically by the method of Givener and Rochefort (7).

Hypothalamic norepinephrine content was determined by a slight modification (26) of the method of Udenfriend and Zaltzman-Nirenberg (24). Plasma TSH levels were determined in triplicate by radioimmunoassay using a National Institute of Arthritis, Metabolism, and Digestive Diseases TSH kit. The assay procedure was according to the directions supplied with assay reagents obtained from the National Institute of Arthritis, Metabolism, and Digestive Diseases Rat Pituitary Hormone Program. The results are expressed as ng of rat TSH-RP-1 (National Institute of Arthritis, Metabolism, and Digestive Diseases) per ml of plasma. Coefficients of variation within assay ranged from 1 to 6.2%.

**Statistics.** Significance of differences between the groups was calculated by Student's t test. All radioimmunoassay data were analyzed on a Wang 2200 computer with Rodbard program.

**RESULTS**

**Experiment A.** The time course of the effects of a large dose of vincristine on corticosterone plasma levels is illustrated in Chart 1. The antitubular agent produced a remarkable adrenocortical activation which had a latent period of 4 hr, reached a peak at 18 hr, and lasted for 48 hr.

The dose-response relationship for the effect of vincristine on plasma corticosterone concentrations is depicted in Chart 2. Vincristine, in an amount as low as 100 µg/kg, increased plasma corticosterone levels significantly (p < 0.05). This appeared to be the minimal effective dose tested, since 50 µg/kg were without effect. Increasing doses of the Vinca alkaloid elevated plasma corticosterone levels in a dose-related manner with 250 µg/kg producing maximal stimulation.

**Experiment B.** Vincristine (250 µg/kg) elicited adrenocortical activation in both normal and hypophysectomized rats (Chart 3). The corticosterone response to vincristine injection was even greater than that which occurred after pharmacological doses of ACTH.

**Experiment C.** Vincristine (1000 µg/kg i.p.) significantly reduced plasma TSH levels 12 and 18 hr after injection (Chart 4).

Vincristine caused a dose-related decrease in the plasma concentration of TSH (Chart 5). The lower doses (50, 100, and 250 µg/kg) of the antimitotic agent did not significantly alter plasma TSH values, whereas the 2 highest doses of vincristine (500 and 1000 µg/kg 18 hr before sacrifice) caused a significant fall of plasma TSH corresponding to 60 and 74% of control values.

Chart 6 illustrates the time course of the effect of the antitubular agent on the hypothalamic content of norepinephrine. The content of this amine remained unmodified at 6, 12, and 18 hr after the injection of vincristine (1000 µg/kg i.p.).

**DISCUSSION**

This is the 1st report of an adrenocortical activation by vincristine, although other microtubular inhibitors such as colchicine, vinblastine, and podophyllotoxin are able to stimulate the secretion of corticosterone in vivo (3) and in vitro (20). The effect of vincristine on adrenal steroid secretion is characterized by a prolonged stimulation which follows a definite latent period. The effect also occurs in hypophysectomized animals, suggesting that it is due to a direct action of vincristine on the adrenal gland.
Other antimitotic agents have been shown to have a direct action on the adrenal gland when tested in vitro (21). Electron microscopic studies (11) have revealed that the Vinca alkaloid causes the disruption and disappearance of microtubules in the adrenal gland. Temple and Wolff (22) have proposed that microtubules restrict access of cholesterol to adrenocortical mitochondria, thereby inhibiting corticosterone biosynthesis. By disrupting the microtubules, vincristine may remove this inhibition and stimulate steroidogenesis of adrenocortical cells. On the other hand, it has been demonstrated that another Vinca alkaloid, vinblastine, increases the cyclic AMP content in the rat adrenal cortex by reducing cyclic AMP phosphodiesterase activity (5). Since cyclic AMP is involved in the adrenal response to ACTH (8) and itself mimics ACTH in stimulating corticosteroidogenesis (9), it is possible that vincristine produces its action on the adrenal cortex by interfering with the metabolism of this cyclic nucleotide.

The adrenal stimulation by vincristine may play some role in the antineoplastic actions of this drug. There are corticosteroid receptors in neoplastic lymphoid tissues (10) so that the effectiveness of vincristine in restricting the growth of these lymphomas may be due, in part, to the actions of the elevated circulating corticosteroids. To test this proposal cortisol levels are currently being determined in patients treated with vincristine.

Although in vitro studies reveal that the anterior pituitary is unresponsive to antimitotic agents (20), the results of the
present in vivo experiments reveal that vincristine decreases TSH release. The mechanism of this inhibition remains to be elucidated. The effect is probably not related to an impairment of the TSH response to TRH because Vinca alkaloids do not inhibit TRH-stimulated release of TSH in vitro (20). Furthermore, a direct stimulation of thyroid hormone release by vincristine, which could, in turn, inhibit TRH-TSH secretion by negative feedback, can be ruled out because Vinca alkaloids inhibit the release of triiodothyronine and thyroxine from thyroid cells (4). Vincristine may interfere with the noradrenergic regulation of TRH-TSH secretion (1). The drug does not alter the steady-state concentration of norepinephrine in the brain (see also Ref. 2). It is possible, however, that vincristine may alter norepinephrine turnover.

On the other hand, since there is evidence that stressful stimuli can depress TSH-thyroid function (15, 16), the possibility that vincristine inhibits TSH release through its toxicity cannot be completely disregarded.

In summary, the present study demonstrates that vincristine increases corticosterone release and decreases TSH release. The adrenocortical response appears to be due to a direct action of vincristine on the microtubules in the adrenal cells. The mechanism underlying the inhibition of TSH release is unknown at the present time.

REFERENCES


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