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Effects of vinblastine on catecholamine-biosynthetic enzymes in heart, sympathetic ganglion and adrenal glands of rats

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Summary

1. The effect of vinblastine on the activities of dopamine-β-hydroxylase (DBH) in heart, superior cervical ganglion and adrenal glands of rats and tyrosine hydroxylase (TH) and phenylethanolamine-*N*-methyl transferase (PNMT) in adrenal glands was examined.

2. In the superior cervical ganglion and heart, DBH activity decreased within hours, reached a minimum in 3 to 5 days and slowly returned towards normal over the next 2 weeks.

3. There was an increase in the activities of TH, DBH and PNMT in adrenal glands which was prevented by adrenal denervation.

4. When the same total dose of vinblastine was administered subdivided over a period of 5 days, enzyme activities in heart, superior cervical ganglion or adrenal glands remained unchanged.

Introduction

Mitotic inhibitors such as vinca alkaloids and colchicine are known to interact with neurotubular protein (Wisniewski, Shelanski & Terry, 1968), and are reported to disrupt neurotubules which appear to be essential for fast axoplasmic transport (Schmitt, 1968). Noradrenaline-containing storage vesicles accumulate in the axons of sympathetic nerves after local treatment with these drugs (Dahlström, 1968, 1970). In rats, Keen & Livingstone (1970) found that a single intravenous injection of vinblastine (3 mg/kg) reduced the noradrenaline content of spleen, heart and vas deferens to 10, 20 and 50% of the control values, respectively. Furthermore, uptake of ^{3}H-noradrenaline is inhibited in isolated perfused rat heart preparations (Keen & Livingstone, 1971).

We have examined the effect of intravenously injected vinblastine on the activity of dopamine-β-hydroxylase (DBH) in heart and superior cervical ganglion and on the activity of tyrosine hydroxylase (TH), DBH and phenylethanolamine-*N*-methyl transferase (PNMT) in adrenal glands. In heart, the effect of vinblastine on storage of ^{3}H-noradrenaline was also checked.

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Methods

Male Sprague-Dawley rats, weighing 150-200 g, received a single intravenous injection of vinblastine sulphate (3 mg/kg) and were killed 1, 2, 3, 5, 15 or 21 days later. The heart, the right superior cervical ganglion and adrenal glands were removed, cleaned, weighed, homogenized and assayed for catecholamine-synthesizing enzymes (TH, DBH and PNMT) as described below. Another group of rats was treated daily with 0·6 mg/kg of vinblastine for 5 days (3 mg/kg total dose), and the animals were killed 3 days after the last injection.

The superior cervical ganglion or both adrenal glands were homogenized in 1 ml of 0·005 M Tris buffer (Tris base), pH 7·5, containing 0·1% Triton X-100 (Research Products International, Inc., Elk Grove Village, Illinois); hearts were homogenized in the same buffer solution (1:5, fresh weight:volume). TH was assayed by a modification of the method of Shiman, Akino & Kaufman (1971). An aliquot of the homogenate of adrenal glands was incubated for 15 min at 37° C with 10 μl of 10^{-3}M L-tyrosine containing 0·25 μCi 3,5-ditritio-tyrosine, 20 μl of 1 M phosphate buffer at pH 6·2, 30 μl of glass-distilled water, 10 μl catalase (1:50 dilution), 10 μl reduced triphosphopyridine nucleotide ($4{\cdot}2 \times 10^{-3}$M) and 10 μl of 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine hydrochloride ($1{\cdot}3 \times 10^{-2}$M). The reaction was terminated by adding 500 μl of 10% trichloroacetic acid. After centrifugation (3,000 rpm), an aliquot of the supernatant was passed over a $0{\cdot}3 \times 1{\cdot}5$ cm column of water-washed Biorad cation exchange resin (AG 50W-X4, H^+ form, 200 mesh) (Calbiochem). The effluent and a 1·5 ml glass-distilled water wash were collected in a scintillation vial and dissolved in 18 ml of Triton X-100 in toluene (1:2) containing 0·015% 1,4-bis[2-(5-phenyloxazolyl)]-benzene and 0·55% 2,5-diphenyloxazole. The tritium was assayed in a Beckman liquid scintillation spectrometer, and the concentration of hydroxylated tyrosine was calculated from the amount of tritiated water found.

DBH was assayed as described by Molinoff, Weinshilboum & Axelrod (1971) with phenylethylamine as substrate. Aliquots (100 μl) of homogenates of superior cervical ganglia, adrenal glands (1:400 dilution) and heart (1:25 dilution) were used, and the phenylethanolamine found was assayed radiometrically with *S*-adenosyl-^{14}C-methionine as methyl donor and purified PNMT as catalyzing enzyme.

PNMT was assayed in the supernatant of the adrenal gland homogenates (100 μl of a 1:25 dilution) as described by Molinoff *et al.* (1971).

In other rats, the effect of vinblastine on storage of ^{3}H-noradrenaline in the heart was examined. The animals received 25 μCi of D,L-7[^{3}H]-noradrenaline intravenously and, one hour later, were given vinblastine (3 mg/kg) or saline intravenously. They were killed 4, 15, 24 or 48 h after drug administration, and levels of labelled and endogenous catecholamines were determined. Catecholamines were adsorbed onto alumina (Woelm, neutral) and eluted with 6 ml of 0·2 N acetic acid. Two ml of the eluate were added to counting vials containing 16 ml Triton X-100-toluene, and the radioactivity was determined by scintillation spectrometry. Endogenous noradrenaline was determined by the trihydroxyindole method as modified by Anton & Sayre (1962).

Drugs used Vinblastine sulphate (Velban, Eli Lilly & Co., Indianapolis, Indiana); desipramine hydrochloride (Petrofrane, Geigy Pharmaceuticals, Ardsley, New York); reserpine (Serpasil, Ciba Pharmaceutical Co., Summit, New Jersey); L-

tyrosine (Calbiochem, Los Angeles, California); 3,5-ditritio-tyrosine (New England Nuclear Corp., Boston, Mass.); catalase (Boehringer Mannheim Corp., New York); reduced triphosphopyridine nucleotide (General Biochemicals, Chagrin Falls, Ohio); 2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine hydrochloride (Aldrich Chemical Co., Milwaukee, Wisconsin).

Results

Administration of a single dose of vinblastine caused rapid loss of body weight, and 30% of the rats died within the first 5 days. The heart weights decreased and the paired adrenal gland weights increased after vinblastine treatment (Table 1).

TABLE 1. *Effect of vinblastine on wet weights (mg) of rat heart and adrenal gland*

Treatment	Heart (mean±S.E.M.)	Paired adrenal glands (mean±S.E.M.)
None (10)	772±23	30±9·0
Vinblastine (10)	583±17*	70±3·4*

Vinblastine (3 mg/kg, i.v.) was injected five days before killing; both treated and untreated animals were killed at the same time. Number of animals is indicated in parentheses. * Effect of vinblastine, significant at $P<0·001$.

Effect of vinblastine on dopamine-β-hydroxylase (DBH) activity in superior cervical ganglion and heart

Two days after giving vinblastine, the DBH activity was decreased by 40% in the superior cervical ganglion and by 57% in the heart. The enzyme activity in the superior cervical ganglion reached a minimum 5 days after vinblastine treatment (23% of control value). In the heart, the DBH activity fell to 22% of the control value three days after drug treatment and remained below control values

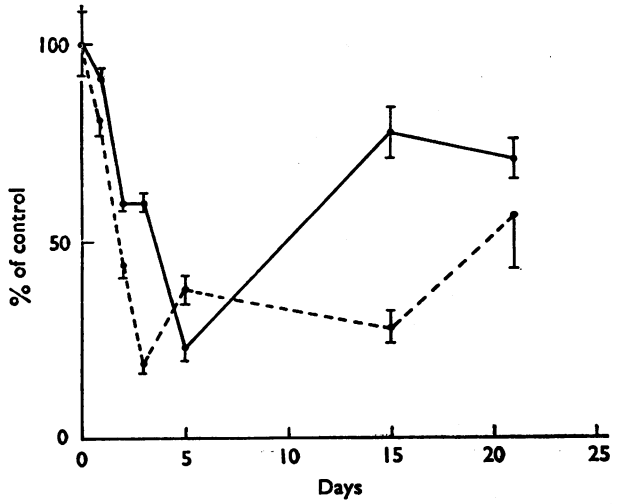

FIG. 1. Time-course of effect of vinblastine (3 mg/kg, i.v.) on the activity of dopamine-β-hydroxylase (DBH) in superior cervical ganglion (●——●) and heart (●- -●) of groups of 6 to 8 rats. In the control rats (100%), the activity of DBH in the superior cervical ganglion was 53·0±8 and in heart 84·6±8 nmol of (product/organ)/hour. Note that the minimum enzyme activity was reached in both organs in 3 to 5 days.

for approximately 15 days. The DBH activity in heart and ganglion partially recovered three weeks after vinblastine treatment, but was still below normal (Fig. 1).

Effect of vinblastine on storage of noradrenaline (Table 2)

Five hours after vinblastine administration, ^{3}H-noradrenaline storage in the heart was normal. After 15 h, the concentration of ^{3}H-noradrenaline in the heart increased when compared with the controls; however, after 48 h, only 9% remained of the ^{3}H-noradrenaline measured in the control heart. The endogenous levels of noradrenaline were unchanged for 24 h and, after 48 h, fell to 24% of those in the controls. To determine whether vinblastine acts on noradrenergic nerve terminals by blocking presynaptic uptake sites for noradrenaline, desipramine (10 mg/kg, i.p.) was injected 20 min prior to vinblastine administration. Forty-eight hours after vinblastine injection, the levels of noradrenaline in control and desipramine-pretreated animals were diminished to the same extent.

TABLE 2. *Effect of vinblastine on storage of exogenous and endogenous noradrenaline (NA) in heart*

Hours after vinblastine	Control	Vinblastine	Control	Vinblastine
	% ^{3}H-NA		ng NA/Heart	
5	100±15	114±43	379±53	396±43
15	100±19	163±21	354±30	288±12
24	100±19	111±24	407±97	451±96
48	100±20	9± 3*	429±77	101±26*

Rats (six/group) were injected intravenously with 25 μCi of ^{3}H-noradrenaline and one hour later were given vinblastine (3 mg/kg) or saline (controls). The animals were killed 5, 14, 24 or 48 h after the last injection. * Effect of vinblastine, significant at $P<0{\cdot}001$.

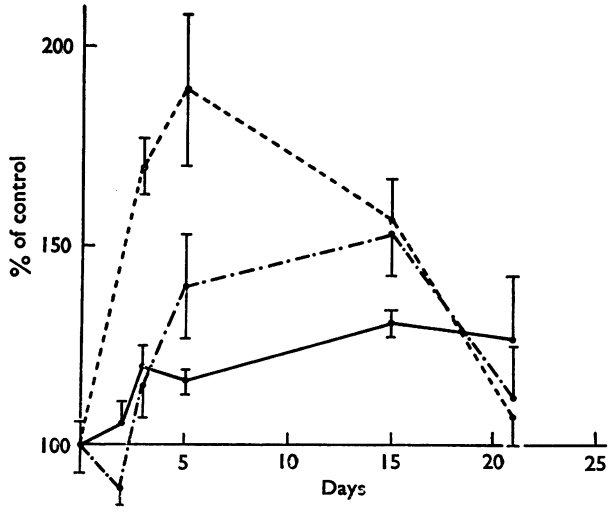

FIG. 2. Time-course of effect of vinblastine (3 mg/kg, i.v.) on activities of tyrosine hydroxylase (TH, ●– – –●), dopamine-β-hydroxylase (DBH, ●–·–·–●) and phenylethanolamine-*N*-methyl transferase (PNMT, ●——●) in adrenal medullae of groups of six to eight rats. The control values (100%) for TH were 1·01±0·06, for DBH 6·72±0·44 and for PNMT 1·15±0·08 (μmol of product/pair of adrenal glands)/hour. Note that the maximum increase in TH activity occurred at 5 days, a time at which DBH and PNMT activities were still increasing.

Effect of vinblastine on adrenal tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine-N-methyl transferase (PNMT) activities

Five days after vinblastine administration, TH activity in the adrenals was nearly doubled and DBH activity was 40% above control values (Fig. 2). After 15 days, TH activity was still elevated by 50% and DBH by 54% above control values. By the 21st day, the activities of both enzymes had returned to control levels. Vinblastine caused a slower and smaller elevation of adrenal PNMT activity; it was maximal after 15 days and returned to control levels after 21 days, as did the activities of the other enzymes.

Effect of unilateral splanchnicotomy on vinblastine-induced increase of adrenal TH, DBH and PNMT activities

Unilateral adrenal denervation caused a decrease of TH activity, but did not affect DBH. The increase in enzyme activities elicited by vinblastine treatment was blocked by denervation (Table 3).

TABLE 3. *Effect of denervation on vinblastine-induced changes in tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) activities in adrenal glands*

	(μmol product/paired glands)/hour			
	TH		DBH	
Treatment	Innervated	Denervated	Innervated	Denervated
None (6)	0·87±0·04	0·46±0·20	7·90±1·20	6·10±1·50
Vinblastine (5)	1·72±0·07*	0·34±0·06	13·40±1·26*	5·80±0·80

Ten days after denervation of the left adrenal gland, vinblastine (3 mg/kg, i.v.) was injected; the animals were killed 5 days after the injection. The number of animals is indicated in parentheses. * Effect of vinblastine, significant at $P<0·001$.

Effect of chronic administration of vinblastine on TH, DBH and PNMT activities in heart and adrenal glands

Administration of daily doses (0·6 mg/kg) of vinblastine over a period of 5 days did not alter DBH activity in the heart, or TH or PNMT activities in the adrenal glands; however, the activity of DBH in the adrenals was significantly decreased by the repeated low doses of vinblastine (Table 4).

TABLE 4. *Enzyme activities in rat adrenal glands and heart after chronic administration of vinblastine*

	(μmol product/organ)/hour			
	Paired adrenal glands		Heart	
Enzyme	Control	Vinblastine	Control	Vinblastine
TH	1·14±0·07	1·07±0·06	—	—
DBH	11·63±0·69	7·21±0·80*	0·088±0·01	0·090±0·01
PNMT	0·83±0·24	0·75±0·06	—	—

Vinblastine (0·6 mg/kg, i.v.) was injected into rats on 5 successive days; control animals were injected with saline. The rats were killed 3 days after the last injection. Tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH), phenylethanolamine-*N*-methyl transferase (PNMT). * Effect of vinblastine, significant at $P<0·01$.

Discussion

Approximately 30 h after the injection of vinblastine, Keen & Livingstone (1970, 1971) found an almost complete disappearance of endogenous noradrenaline in peripheral sympathetic nerves of rats. Decreased noradrenaline concentrations in tissues can be attributed to one of the following mechanisms: (1) inhibition of vesicular storage; (2) displacement of noradrenaline from its storage sites; (3) blockade of axonal transport of both storage granules and noradrenaline-synthesizing enzymes; (4) reduction of the activity of catecholamine-biosynthesizing enzymes.

If vinblastine were acting on the storage process by a mechanism comparable to that of reserpine, the rate of noradrenaline depletion by both drugs should be similar. However, vinblastine reduces cardiac noradrenaline concentration by 74% in 48 h while reserpine depletes it by 50% in 16 min (Costa, Boullin, Hammer, Vogel & Brodie, 1966).

Drugs which displace noradrenaline from its storage sites are accumulated in noradrenergic nerves by a specific uptake mechanism (Stone, Porter, Stavorski, Ludden & Totaro, 1964) which is blocked by a number of compounds including desipramine. Because our experiments show that pretreatment with desipramine does not counteract the effects of vinblastine on cardiac noradrenaline concentrations, it is suggested that displacement of noradrenaline is not involved in its depletion by vinblastine.

Blockade of axonal transport and/or inhibition of protein synthesis could reduce the activity of enzymes involved in catecholamine biosynthesis in nerve endings. Moreover, reduction of the number of synaptic vesicles as a result of axonal transport blockade (Dahlström, 1970) could also cause a diminution of noradrenaline concentrations. Vinblastine may interfere with protein synthesis (Creasy & Markiw, 1964). The present experiments show that after vinblastine treatment, DBH in the heart is reduced by 50% in about 20 h whereas it takes 80 h to reduce it to the same extent in sympathetic ganglia. If axonal transport is blocked, there should be a decrease of DBH in the heart and an accumulation of the enzyme in sympathetic ganglia. If protein synthesis were inhibited, the decrease in DBH elicited by vinblastine should be most rapid in the ganglion. In ganglia, DBH has a half-life of 15 h (Axelrod, 1972). Our results do not exclude an effect on protein synthesis since the decrease in enzyme levels in the ganglia could be slowed by inhibition of transport. Vinblastine-induced depletion of heart noradrenaline could be due to reduction of noradrenaline synthesis.

After vinblastine, the activities of TH, DBH and PNMT in adrenal glands were increased but returned to normal when DBH activities in heart and superior cervical ganglion were restored. From these findings we can infer that, in adrenals, the synthesis of catecholamines is enhanced possibly as a compensatory response to the reduction of noradrenaline synthesis in peripheral sympathetic neurones. This compensation is mediated trans-synaptically since it was abolished by unilateral splanchnicotomy. The steady-state level of adrenaline and noradrenaline in adrenal glands is probably maintained in vinblastine-treated rats by enhanced synthesis to compensate for enhanced release of the catecholamine (Cheney, Hanin, Massarelli, Trabucchi & Costa, 1972). In heart and adrenal glands of rats receiving small chronic doses of vinblastine, neither the enzyme activities nor catecholamine concentrations were changed; however, the mortality of the rats was markedly in-

creased. Vinblastine causes an arrest of dividing cells in the metaphase (Cardinali, Cardinali, Handler & Agrifoglio, 1971; Boggs, Athens, Haab, Cancilla, Raab, Cartwright & Wintrobe, 1964); therefore, the destruction of proliferating cells in both bone marrow and the gastrointestinal tract might have led to the increased mortality. The toxicity of small chronic doses of vinblastine is more likely to be a result of its effect in tissues with fast-dividing cells than of its effect in adrenergic neurones.

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