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EFFECTS OF VINBLASTINE ON NORADRENERGIC AXONS

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1 The effects of vinblastine and 6-hydroxydopamine (6-OHDA) on various parameters of adrenergic neurone functions in the rat were examined and compared.

2 During the first 12 h after injection of vinblastine, although the concentration of cardiac noradrenaline was unaltered, the turnover rate of the catecholamine in the heart was reduced to 40 ng g^{-1} h^{-1} compared to a rate of 66 ng g^{-1} h^{-1} in the hearts of animals treated with 0.9% w/v NaCl solution.

3 The uptake of noradrenaline by rat atria *in vitro* was inhibited by vinblastine (2.5×10^{-4} M) or 6-OHDA (5×10^{-5} M), but only the inhibitory effect of vinblastine was readily reversible. The *in vitro* uptake of noradrenaline by atria of vinblastine- or 6-OHDA-treated rats was found to be impaired two days after injection of the drugs. After 14 days, however, the noradrenaline uptake was 71% of control values after vinblastine and 91% after 6-OHDA.

4 In addition to intensely fluorescent nerve trunks in epicardial connective tissue septa, a decrease in the number of fluorescent adrenergic terminals in the atria and ventricles could be observed two days after vinblastine injection. It is suggested that intravenous vinblastine treatment, like 6-OHDA, results in the destruction of adrenergic nerve terminals.

Introduction

Local application of vinblastine to either sympathetic ganglia or axons results in alteration of the morphology, biochemistry and function of adrenergic neurones, presumably as a consequence of the impairment of axoplasmic flow (Dahlström, 1968, 1971; Schmitt, 1968; Kreutzberg, 1969; Perisic & Cuenod, 1972). After intravenous injection of vinblastine, the dopamine-β-hydroxylase activity is reduced in sympathetic ganglia as well as in adrenergic nerve endings in the heart (Hanbauer, Kopin, Maengwyn-Davies, Thoa & Weise, 1973). Thus, the reduction in activity of the enzyme cannot be due exclusively to a blockade of axonal transport. Furthermore, the studies of Keen & Livingstone (1970b, 1971) on depletion of noradrenaline after administration of vinblastine reveal a number of inconsistencies with the concept that systemically administered vinblastine impairs axonal transport. In fact, these authors concluded that, although the overall effect of vinblastine is a blockade of noradrenaline synthesis in peripheral axons, a relation between impairment of axonal flow and inhibition of noradrenaline biosynthesis could not be established.

The following experiments were carried out to examine further the actions of intravenously administered vinblastine. The selective action of vinblastine on terminal axons of noradrenergic neurones and its lack of action on cholinergic nerves (as reported by Cheney, Hanin, Maosarelli, Trabucchi & Casta, 1973), is similar to the effects of 6-hydroxydopamine (6-OHDA). In the present study, the effects of vinblastine and of 6-OHDA on various parameters of noradrenergic neurone function were examined and compared.

Methods

Estimation of the rate of noradrenaline turnover in rat heart

Male Sprague-Dawley rats weighing 180-200 g (Zivic-Miller Laboratories, Allison Park, Pennsylvania) were given vinblastine (3 mg/kg) intravenously. Thirty minutes later, the animals received (−)-[7-^{3}H]-noradrenaline (50 μCi/kg, i.v.) dissolved in 1 ml 0.9% w/v NaCl solution (saline) containing 1 mg ascorbic acid and adjusted to pH 5.5. The rats were decapitated 3, 6, 9 or 12 h after administration of [^{3}H]-noradrenaline; the hearts were removed, weighed, frozen on dry ice and stored at −20°C until analysed. The following day, the hearts were homogenized in 2 ml 0.4 M perchloric acid containing 0.05% sodium metabisulphite. After centrifugation, the noradrenaline

in the supernatant fraction was adsorbed onto aluminium oxide (Alumina Woelm, neutral) at pH 8.6 and eluted with 6 ml 0.2 N acetic acid. The tritium content of 2-ml portions of the eluate was assayed by liquid scintillation spectrometry after dissolving in 16 ml Triton X-100 : toluene (1 : 2). Another portion was used to determine the endogenous noradrenaline by a modification (Weil-Malherbe & Bigelow, 1968) of the trihydroxyindole method of Anton & Sayre (1962). The specific activity of noradrenaline was calculated from the tritiated and endogenous amines present and the turnover rate for noradrenaline was estimated from the exponential decline of the specific activity, as described by Costa & Neff (1970).

Measurement of uptake of noradrenaline by rat atria in vitro

Rats were injected with vinblastine (3 mg/kg, i.v.) and killed 2, 7, 14 and 21 days after injection of the drug. The atria were incubated at 37° C in an atmosphere of 95% oxygen and 5% carbon dioxide with 10^{-7} M (±)-[7-^{3}H]-noradrenaline in Krebs-Ringer bicarbonate buffered at pH 7.4. To remove nonspecifically bound amines from the tissues, incubation was continued for an additional 10 min in a medium of the same composition, but which contained no radioactive noradrenaline. The atria were then homogenized in 0.5 ml 0.8 M perchloric acid. After centrifugation of the homogenate, a portion of the clear supernatant was added to 16 ml Triton X-100 : toluene mixture (1 : 2) for determination of total radioactivity by liquid scintillation spectrometry.

Fluorescence histochemistry

The fluorescence of atrium and ventricle slices, and of flat iris preparations, was studied at various times after vinblastine injections by the catecholamine histochemical method of Falck and Hillarp (Falck, 1962; Falck, Hillarp, Thieme & Torp, 1962).

Drugs

Vinblastine (Velban) was obtained from Eli Lilly and Company (Indianapolis, Indiana); 6-hydroxydopamine hydrochloride from Regis Chemical Company (Chicago, Illinois); and (±)-[7-^{3}H]-norepinephrine (specific activity 10 Ci/mmol) from New England Nuclear Corporation (Boston, Massachusetts).

Results

Estimation of turnover rate of cardiac noradrenaline after vinblastine

Cardiac noradrenaline concentrations remained unchanged during the first 12 h after invtravenous administration of vinblastine (3 mg/kg), but the concentrations of radioactive noradrenaline declined (Table 1). The rate of decline of the specific activity was estimated by regression analysis of the data plotted in Figure 1. The equation for the decline of the specific activity (sp. act.) of cardiac [^{3}H]-noradrenaline of saline-treated rats is sp. act. = $730e^{-0.13t}$ and that of vinblastine-treated rats is sp. act. = $732e^{-0.084t}$ Statistical analysis revealed a significant difference ($P < 0.050$) between the two slopes (Burlington & May, 1970). From the fractional rate constant for the decline in specific activity of cardiac noradrenaline and from the endogenous noradrenaline content, the turnover rate of cardiac noradrenaline in vinblastine-treated rats was calculated to be 40 ng g^{-1} h^{-1} while that of saline-treated rats was 66 ng g^{-1} h^{-1}.

Table 1 Concentrations and specific activity of noradrenaline (NA) in rat heart ventricles at various times after vinblastine administration.

	Saline (i.v.)		*Vinblastine (3 mg/kg, i.v.)*	
*Hours after (–)-[7-^{3}H]-NA**	*ct min^{-1} ng^{-1} ±s.e. mean*	*NA ng/g heart ±s.e. mean*	*ct min^{-1} ng^{-1} ±s.e. mean*	*NA ng/g heart ±s.e. mean*
3	500 ± 41	470 ± 19 (6)**	550 ± 36	480 ± 24 (9)
6	330 ± 19	540 ± 41 (9)	510 ± 17†	470 ± 28 (10)
9	230 ± 15	470 ± 30 (4)	330 ± 27†	440 ± 53 (4)
12	160 ± 6	530 ± 38 (10)	260 ± 9†	540 ± 36 (11)

* 10 μCi (–)-[7-^{3}H]-Noradrenaline in 1 ml saline containing 1 mg ascorbic acid (pH 5.5) was injected intravenously 30 min after injection of vinblastine. **Number of experimental animals is indicated in parentheses. Comparison with corresponding control value: †$P < 0.001$.

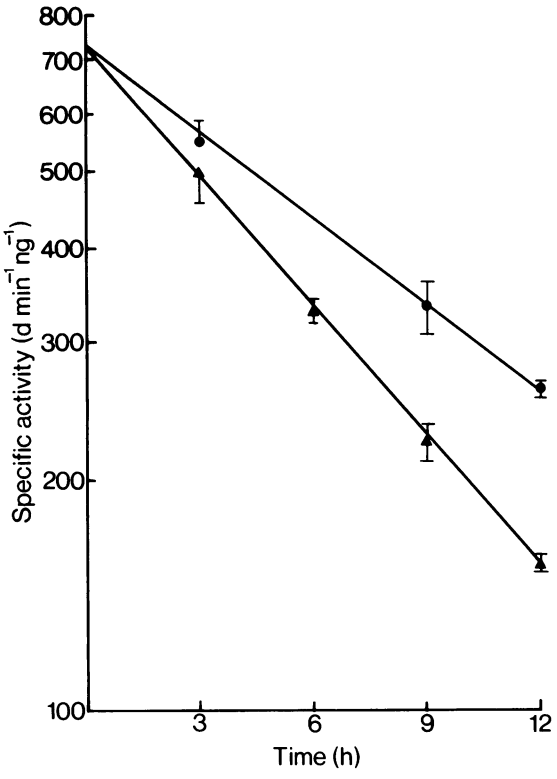

Fig. 1 Time course of the specific activity (d min^{-1} ng^{-1}) of noradrenaline in heart ventricles of rats treated with vinblastine (3 mg/kg, i.v.) 30 min prior to i.v. injection of 10 μCi (−)-[^{3}H]-noradrenaline. ●, saline-treated rats (k = 0.084 h^{-1}); and ▲, vinblastine-treated rats (k = 0.13 h^{-1}).

In vitro *uptake of [^{3}H]-noradrenaline by heart tissue*

The uptake of [^{3}H]-noradrenaline by rat heart atria was inhibited by a high concentration (2.5×10^{-4} M) of vinblastine (Table 2). However, the inhibition of uptake of [^{3}H]-noradrenaline by vinblastine is readily reversible. Atria incubated with vinblastine (2.5×10^{-4} M) for 30 min rapidly regain the normal capacity to take up noradrenaline when vinblastine is rinsed out of the tissue (Table 2). 6-OHDA inhibited uptake of [^{3}H]-noradrenaline by atria in lower concentrations (5×10^{-5} M) than did vinblastine, and its effects on noradrenaline uptake sites were irreversible when the drug was washed out of the tissue.

In vitro *uptake of [^{3}H]-noradrenaline in tissues of vinblastine-treated rats*

Two days after injection of vinblastine (3 mg/kg, i.v.), the atria of rats appeared to have lost almost totally their ability to take up noradrenaline. Seven days following vinblastine injection, however, noradrenaline uptake was partially restored. The rapid recovery of noradrenaline uptake which occurred during the first week did not result in complete restoration of [^{3}H]-noradrenaline uptake. Thirty days after vinblastine injection, uptake of the amine was still inhibited by about 34%. Uptake of noradrenaline by atria of 6-OHDA-treated rats (20 mg/kg, i.p.) was blocked two days after drug administration but began to return towards normal after one week and had almost completely recovered after two weeks (Table 3). However, vinblastine administration did not appear to affect uptake of noradrenaline by the iris.

Table 2 Noradrenaline uptake by rat atria in the presence of vinblastine or 6-hydroxydopamine.

	Noradrenaline pmol/g tissue ± s.e. mean	
Drug concentration	*Before drug washout**	*After drug washout***
Krebs-Ringer (pH 7.4)	630 ± 51	–
Vinblastine (10^{-4} M)	380 ± 24†	523 ± 66
Vinblastine (2.5×10^{-4} M)	89 ± 6††	471 ± 57
6-Hydroxydopamine (5×10^{-5} M)	174 ± 12††	115 ± 8††

*Atria were preincubated for 30 min in media containing 6-hydroxydopamine or vinblastine. Thereafter, they were incubated for 30 min in media containing the drug and [^{3}H]-noradrenaline. **Atria were preincubated for 30 min in media containing 6-hydroxydopamine or vinblastine. Thereafter, they were incubated for 15 min in Krebs-Ringer medium (washout) followed by incubation for 30 min with [^{3}H]-noradrenaline. Comparison with control value: †Degrees of freedom = 6; $P < 0.01$; ††Degrees of freedom = 6; $P < 0.001$.

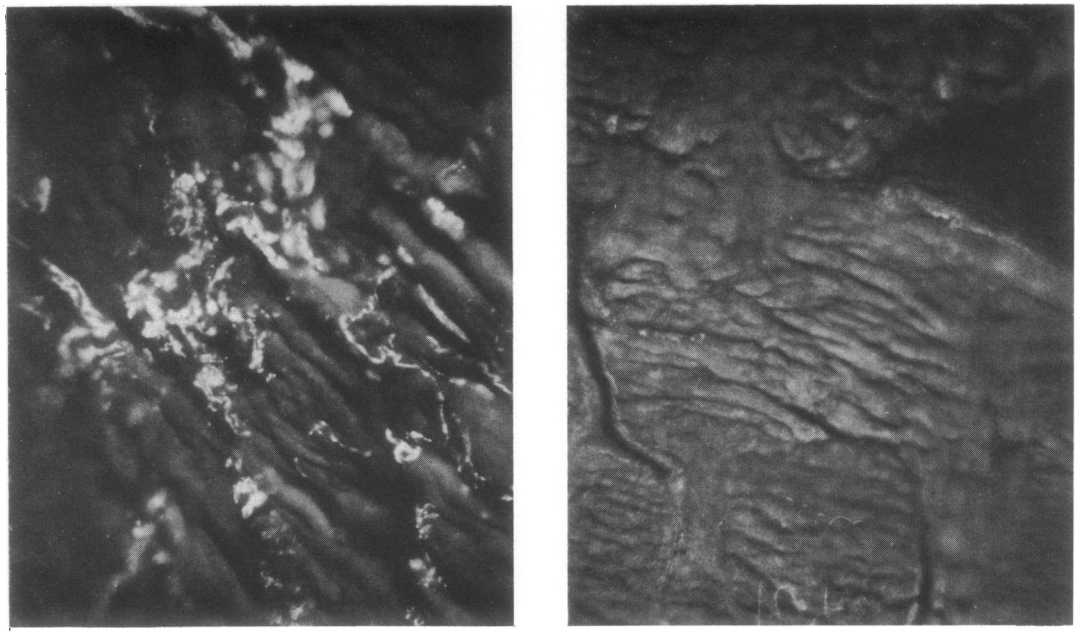

Fig. 2 Rat atrium (x190). (a) Control atrium. Normal adrenergic nerves. (b) Vinblastine-treated atrium (2.5 mg/kg, i.v., two days). No nerve fibres were observed.

Fluorescence histochemistry of atria and irises

Two days after administration of vinblastine (2.5 mg/kg, i.v.), a large decrease in the number of fluorescent adrenergic nerves was observed in the atria (Fig. 2) and ventricles (Fig. 3) with only a few fibres remaining. The yellow fluorescent mast cells containing 5-hydroxytryptamine were not affected by the drug. A few intense fluorescent nerve trunks were observed coursing in the connective tissue septa in the epicardium (Figure 4). No changes were observed in the irides or superior

Table 3 Effects of administration of vinblastine and 6-hydroxydopamine on [^{3}H]-noradrenaline uptake by rat atrium and iris *in vitro*.

	Noradrenaline uptake		
	Atrium		*Iris*
Treatment	*(pmol/g ± s.e. mean)*	*% Inhibition*	*(pmol/g ± s.e. mean)*
Saline (i.v.)	550 ± 17 (9)	0	202 ± 20 (6)
Vinblastine (3 mg/kg, i.v.)			
2 days	53 ± 12 (4)*	91	176 ± 14 (6)
7 days	320 ± 46 (5)*	42	–
14 days	390 ± 27 (6)*	29	–
21 days	340 ± 21 (10)*	38	–
30 days	360 ± 33 (6)*	34	–
Saline (i.v.)	630 ± 30 (4)	0	–
6-Hydroxydopamine (20 mg/kg, i.v.)			
2 days	70 ± 7 (4)*	81	–
7 days	160 ± 20 (4)*	75	–
14 days	501 ± 39 (4)	20	–

Number of determinations is indicated in parentheses. Comparison with corresponding control value: *$P < 0.01$.

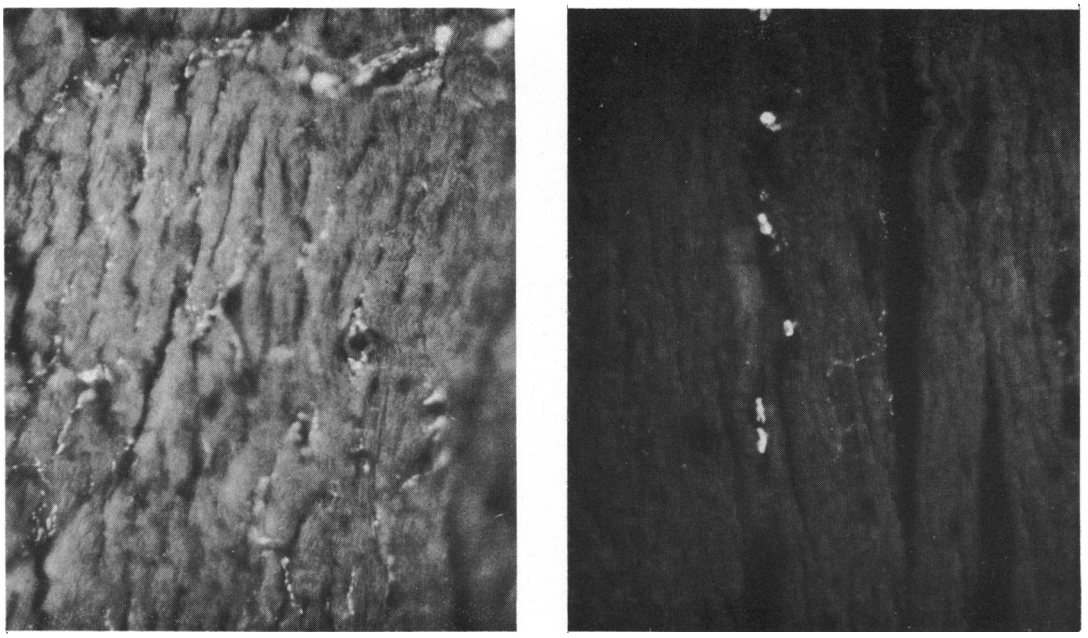

Fig. 3 Rat ventricle (x190). (a) Control ventricle. Normal nerves and mast cells. (b) Vinblastine-treated ventricle (2.5 mg/kg, i.v., two days). Sparse nerves were observed. Normal fluorescent mast cells were also present.

cervical ganglia four or 14 days after injection of vinblastine (2.5 mg/kg, i.v.).

Discussion

The present *in vitro* and *in vivo* results demonstrate that vinblastine and 6-OHDA affect noradrenaline uptake in noradrenergic terminal axons by different mechanisms. Both drugs antagonize noradrenaline uptake *in vitro*, but the concentration of vinblastine necessary to block noradrenaline uptake by rat atria was about 25-fold higher than that of 6-OHDA. The inhibitory effect of vinblastine disappeared after the drug was removed from the bath; in a similar experiment, however, the blocking effect of 6-OHDA was not readily reversible (Table 2). Desipramine (10 mg/kg, i.p.), which inhibits amine uptake by sympathetic neurones (Glowinski & Axelrod, 1964), administered 60 min before injection of vinblastine, fails to block the drug-induced depletion of noradrenaline in the heart (Hanbauer *et al.*, 1973). Desipramine, however, prevents the depletion of cardiac noradrenaline content by 6-OHDA (Stone, Porter, Stavorski, Ludden & Totaro, 1964). Another important difference related to the mode of action of vinblastine and 6-OHDA is the time course of

Fig. 4 Rat atrium (x75), vinblastine-treated (2.5 mg/kg, i.v., two days). Intense fluorescent adrenergic nerve trunks.

cardiac noradrenaline depletion. After injection of 6-OHDA (20 mg/kg, i.v.), the concentration of noradrenaline in the heart was found to be decreased within 2.5 h (Goldman & Jacobowitz,

1971; Jonsson & Sachs, 1972); after vinblastine administration (3 mg/kg, i.v.), the noradrenaline content of the heart did not appear to decline until after 24 h (Keen & Livingstone, 1970a). Cheney *et al.* (1973) have interpreted this delayed action of vinblastine as related to the formation of a vinblastine metabolite responsible for its action on tissue noradrenaline concentrations. In contrast, the present experiments show that after vinblastine administration, although the uptake of [^{3}H]-noradrenaline remains unaltered, the turnover rate of cardiac noradrenaline is decreased between 3 and 12 h after its administration.

Poisner & Bernstein (1971) and Thoa, Wooten, Axelrod & Kopin (1972) showed that vinblastine or colchicine in concentrations ranging from 10^{-3} M to 5×10^{-5} M inhibited the noradrenaline release by nerve impulses. In agreement with this view, Perisic & Cuneod (1972) considered that the depression of postsynaptic responses recorded in the tectum of the pigeon after intraocular injection of colchicine was due to a blockade of synaptic transmission. Inhibition of release of noradrenaline by vinblastine would be consistent with the diminished amine turnover observed before the decline in noradrenaline content.

The appearance of intensely fluorescent nerve trunks and the decrease in fluorescence of adrenergic nerves in the heart are similar to the effects observed after 6-OHDA treatment (Goldman & Jacobowitz, 1971). Previous studies have pointed out large variations in tissue susceptibility to 6-OHDA (Thoenen & Tranzer, 1968; Haeusler, Haefely & Thoenen, 1969; Goldman & Jacobowitz, 1971). It is believed that differences in blood flow could account for unequal effects on noradrenaline content in various tissues such as the heart, iris and vas deferens. Similarly, in the present study, the absence of an influence of vinblastine on adrenergic neurones in the iris could be attributed to the relatively small blood flow into the iris as compared to its high density of adrenergic neurones. The depletion of noradrenaline in the heart and the prolonged inability to take up [^{3}H]-noradrenaline suggests that vinblastine, like 6-OHDA, causes degeneration of adrenergic terminals. It would appear that a biphasic effect occurs after vinblastine injection, i.e. an initial reduction of impulse transmission, which would explain the decrease in noradrenaline turnover, followed by degenerative processes in the nerve terminals. A partial return of the ability of rat atria to take up [^{3}H]-noradrenaline after 14 days is also observed after 6-OHDA since regenerative processes appear to have taken place.

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