Selective Noradrenergic Denervation of the Heart Following Intravenous Injections of Vinblastine or Vincristine

T. BENNETT1, SHEILA M. GARDINER2, and D. R. TOMLINSON1

1 Department of Physiology and Pharmacology, University Hospital and Medical School, Clifton Boulevard, Nottingham, NG7 2UH, Great Britain
2 Department of Physiology and Environmental Studies, School of Agriculture, University of Nottingham, Sutton Bonington, Nr. Loughborough, Leicestershire, Great Britain

Summary. 1. The progressive effects of a single injection (3 mg/kg i.v.) of either vinblastine or vincristine on the innervation of the rat atria have been examined.
2. Functional studies were performed by stimulating electrically the noradrenergic or cholinergic nerve fibres within isolated left atria from treated and control rats. In addition, the effects of the drugs on the ultrastructure of both types of nerve fibre were examined. The noradrenergic innervation was further examined by fluorescence histochemistry and measurement of the noradrenaline content of the atria.
3. On the basis of these studies it appeared that both drugs caused degeneration of noradrenergic nerves; cholinergic nerves seemed to be unaffected. Marked effects were seen 48 h after vinblastine treatment or 30 h after vincristine treatment.
4. Evidence is presented and discussed as to whether or not this action of the vinca alkaloids can be ascribed to their well-established effects on microtubules and intra-axonal transport processes.

Key words: Vinca alkaloids — Heart — Noradrenergic denervation.

INTRODUCTION

Keen and Livingston (1970, 1971) were the first to demonstrate that an i.v. injection of vinblastine (3 mg/kg) into rats caused a depletion of endogenous levels of noradrenaline. These effects were most marked 2 days after drug administration, but Keen and Livingston (1971) indicated that the changes were not likely to be due to neuronal degeneration.

More recent studies have confirmed the above biochemical findings (Hanbauer et al., 1973; Hanbauer et al., 1974) but it now seems probable that the effects of i.v. administered vinblastine are due to degeneration of noradrenergic axons (Bennett et al., 1973; Hanbauer et al., 1974).

It has been claimed that the action of vinblastine on noradrenergic neurones is not shared by its chemical analogue, vincristine (Cheney et al., 1973), but it is important to note that Cheney et al. (1973) investigated only the effects of low doses of vincristine (0.6 mg/kg) and compared them with the effects of high doses of vinblastine (3 mg/kg). These workers produced evidence that neither vinblastine nor vincristine, in the doses used, reduced acetylcholine levels in the heart, or a variety of other tissues—the implication being that neither drug affected cholinergic nerves.

The present study is concerned with a variety of aspects of the autonomic innervation of the rat heart following intravenous administration of vinblastine or vincristine. A preliminary account of some of the results has been given (Bennett and Gardiner, 1975).

METHODS

Male Wistar rats weighing between 250 and 280 g were used throughout the study. Vinblastine ("Velbe", Lilly) or vincristine (" Oncovin", Lilly) were dissolved in sterile saline and injected into a tail vein. Vinblastine was administered at a dose of 3 mg/kg and vincristine at a dose of 3 mg/kg or 0.6 mg/kg; the treated animals and un.injected control animals were kept under identical conditions. The treated animals were killed 24, 30 or 48 h after injection of vinblastine; 24 or 30 h after injection of vincristine (3 mg/kg) and 48 h after administration of vincristine (0.6 mg/kg). Animals injected with 3 mg/kg vincristine did not survive for 48 h.

The hearts were removed from control and treated animals and the following investigations carried out.

Organ Bath Studies. The left-atrium was suspended between parallel platinum wire electrodes in a 100 ml jacketed organ bath containing a physiological saline solution of the following composition: NaCl, 118 mM; KCl, 4.7 mM; CaCl2, 2.5 mM; MgSO4, 2.4 mM; NaHCO3, 30 mM; Na2HPO4, 1 mM; glucose, 11.1 mM. The solution was kept at 37°C and bubbled with 95% O2, 5% CO2.
The quiescent atrial tissue was electrically driven by field stimulation via the platinum wire electrodes for the period of the experiment. The stimulus parameters used (3 Hz, 2 ms pulse width, 8–10 V strength) had little or no effect on the intramural nerves of the atrium. In order to excite these nerves, the stimulus strength was increased to 100 V for a period of 15 s; the nerve-mediated effects were seen as a change in inotropy of the driven atrium (Blinks, 1960; Bolton, 1967). In those experiments in which the responses to noradrenergic nerve stimulation, noradrenaline or isoprenaline were examined, atropine (5 x 10⁻⁶ M) was present throughout; propranolol (1 x 10⁻⁶ M) was present throughout those experiments in which the responses to cholinergic nerve stimulation or acetylcholine were investigated. The contractions of the tissue were recorded through an isometric transducer (SR1) connected to a flat-bed pen recorder (Servoscribe). The system was calibrated against a mass of 1 g and the results given in the text refer to the force of contraction developed. Tissues were allowed to equilibrate for 30 min before any measurements were made.

**Endogenous Noradrenaline Content.** Right and left atria were excised from freshly killed rats, 48 h after vinblastine and 30 h after vincristine treatment; they were blotted dry, weighed and homogenised in 1 ml of 5% perchloric acid. Each homogenate was centrifuged at 15000 x g for 20 min at 4°C. The supernatant was retained, the sediment extracted with a further 1 ml of 5% perchloric acid, and the resultant suspension recentrifuged. The two supernatants thus obtained from each pair of atria were pooled. The extracts were then assayed for noradrenaline by the method of Hjäggendal (1963) with the single modification that the fluophores were stabilised by the addition of 0.15 ml glacial acetic acid 5 min after addition of the NaOH (Valori et al., 1970).

The fluorescence of the samples, standards and blanks was measured in an Aminco-Bowman spectrofluorimeter. The difference in fluorescence emission (at 480 nm) between each sample and its faded blank (see Hjäggendal, 1963) was used to determine the noradrenaline content by interpolation on a standard curve.

**Ultrastructural Studies.** Atrial tissue was examined with the electron microscope after fixation by either of two methods. A general ultrastructural appraisal was made using material fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3 followed by electron intensification in osmium tetroxide. More specific information about the noradrenergic nerves was obtained using material fixed in acrolein and sodium dichromate (Woods, 1969). The fixation of noradrenergic nervous tissue by these two methods has been compared in detail by Tomlinson (1975).

After fixation, tissue blocks were dehydrated in graded ethanolis, passed through epoxypropane and embedded in epoxy-resin. Sections were cut with a Reichert OUM 2 ultramicrotome, stained on the grid with aqueous lead citrate (Reynolds, 1963) and examined in a Philips EM 300 electron microscope.

In addition to a straightforward examination of neuronal ultrastructure, an attempt was made to determine whether vinblastine treatment interfered with the membrane uptake mechanism for catecholamines (see Iversen, 1967) in noradrenergic neurons. Animals injected with vinblastine (3 mg/kg) 47 h previously were injected i.v. with 200 mg/kg 5-hydroxydopamine (Hassle) 1 h before being killed. Under normal conditions, 5-hydroxydopamine is taken up by noradrenergic axons and is concentrated in the storage vesicles, greatly enhancing their electron density (Tranzer and Thoenen, 1967). Atria from animals treated in this way were compared with atria from control animals injected with 200 mg/kg 5-hydroxydopamine 1 h before death. A qualitative comparison was made of the extent of vesicle granulation in the two situations.

A major problem in the ultrastructural assessment of sympathetic denervation arises from the fact that profiles of intervaricosities of noradrenergic neurones cannot be distinguished from those of cholinergic neurones because such profiles contain few or no vesicles. We attempted to overcome this problem by injecting rats with 6-hydroxydopamine, which produces a specific degeneration of noradrenergic axons (Tranzer and Thoenen, 1967). Subsequent treatment with vinblastine and vincristine permits the drugs to act only on cholinergic neurones in atria and degeneration of these fibres can be searched for.

Rats were therefore injected with a large dose of 6-hydroxydopamine (250 mg/kg i.p.) and left for 5 days to permit degeneration of the noradrenergic nerves supplying the right atrium. At this time half the animals were injected with either vinblastine or vincristine (3 mg/kg i.v.) and left for 48 or 30 h (respectively) before removal of the right atria for fixation in glutaraldehyde followed by osmication. Atria from control animals (treated only with 6-hydroxydopamine) were also taken after similar time intervals.

**Drugs.** The following drugs were used in this study: vinblastine sulphate ("Velbe", Lilly); vincristine sulphate ("Oncovin", Lilly); 5-hydroxydopamine hydrobromide (H85/35; Hassle); 6-hydroxydopamine hydrochloride (H88/32; Hassle); l-noradrenaline bitartrate (Sigma); isoprenaline hydrochloride (Moore); acetylcholine chloride (BDH); atropine sulphate (BDH); and propranolol hydrochloride (ICI). The values given for the latter 5 drugs are in terms of the base, figures cited for the others refer to the salts.

**Treatment of Results.** Numerical results in the text are given as the mean value ± 1 standard error of the mean; n is the number of experiments (also equal to the number of animals). Results were analysed for statistical significance using Student's unpaired 't'-test.

**RESULTS**

Before the animals were killed they were examined for any outward signs of the effects of vinblastine or vincristine. At the higher dose level (3 mg/kg) both drugs produced gait disturbances, although most of the time the animals appeared sedated and maintained an unnaturally hunched posture, with their eyes closed. Respiration was rapid and shallow, and there was marked piloerection. The animals usually had a crust of blood around the nostrils, and those given 3 mg/kg vincristine had diarrhoea.

**Functional Studies on Isolated Atria from Vinblastine Treated Rats.** In the presence of atropine (5 x 10⁻⁶ M) left atria from control animals responded to noradrenergic nerve stimulation at 3 Hz with a positive inotropy (Table 1). Twenty four and 30 h after injection of vinblastine the atria showed a significant depression of the response to noradrenergic nerve stimulation (Table 1). By 48 h after vinblastine treatment the positive inotropic response to noradrenergic nerve stimulation was undetectable (Table 1).

Log dose/response curves for the positive inotropic effect of noradrenaline were obtained for atria from control and experimental animals and the dose at which the response was 50% of the maximum (ED₅₀) was found by interpolation. The results for atria from animals injected 24 and 30 h previously with vinblastine were pooled. The dose response curve showed a parallel displacement relative to the control; this change represented a 12-fold increase in sensitivity to...