Nicotinic and Nonnicotinic Receptor-Mediated Actions of Vinblastine (43614)

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Abstract. **Vinblastine has been demonstrated to inhibit nicotinic acetylcholine receptor (nAChR) activity in adrenal chromaffin cells and superior cervical ganglia and to alter agonist binding affinity to nAChR of the electric organ of *Torpedo californica*. In cultured chromaffin cells, vinblastine (IC_{50}, 8.9 μM) is significantly more potent than hexamethonium (IC_{50}, 16 μM) and decamethonium (IC_{50}, 18 μM) and significantly less potent than *d*-tubocurarine (IC_{50}, 2 μM), pentolinium (IC_{50}, 0.6 μM), and mecamylamine (IC_{50}, 0.1 μM) in inhibiting nAChR-stimulated catecholamine release. These results demonstrate that vinblastine has moderately potent anti-nAChR activity on adrenal nAChR. On the other hand, vinblastine does not interfere with phrenic nerve stimulation of rat diaphragm musculature in concentrations up to 200 μM. However, in relatively high doses, vinblastine (10–200 μM) produces an increase in baseline tension of diaphragm muscle. This effect is concentration related (EC_{50}, ~88 μM), reversible, and independent of phrenic nerve stimulation. The elevation in baseline tension is unaffected by nAChR blockade via *d*-tubocurarine, but is dependent upon the presence of extracellular calcium. The results suggest that vinblastine's antinicotinic actions are selective for neuronal-type nAChR and do not extend to nAChR of mammalian skeletal muscle. High concentrations of vinblastine appear to elicit contractures of skeletal muscle that are unrelated to nAChR.** [P.S.E.B.M. 1993, Vol 203]

Vinblastine, a therapeutically useful antineoplastic drug, interferes with cell mitosis by promoting the depolymerization of spindle fiber microtubules (1). In addition, vinblastine has been demonstrated to inhibit nicotinic acetylcholine receptor (nAChR)-stimulated catecholamine release from perfused bovine adrenal gland (2, 3) and cultured bovine adrenal chromaffin cells (4), to block transmission through the cat superior cervical ganglion (2), and to affect binding properties of nAChR-rich membrane preparations from the electric organ of *Torpedo californica* (5). It is not known whether the actions of vinblastine on nAChR are related to actions of vinblastine on microtubules, although evidence in the literature suggests that such a possibility exists in cultured adrenal chromaffin cells (4, 6, 7). However, it has also been suggested that the antimitotic and nAChR actions in these tissues are unrelated (2, 5).

Although vinblastine's inhibitory effects on ganglionic transmission and its inhibition of nAChR-stimulated release of catecholamines from adrenal glands and adrenal chromaffin cells are documented, it is not known whether these effects can be generalized to include anticholinergic activity on nAChR of mammalian skeletal muscle. The objectives of the studies reported here were (i) to compare the antinicotinic actions of vinblastine on the adrenal nAChR to those of several classical neuromuscular and ganglionic blockers and (ii) to determine whether vinblastine disrupts nerve transmission at the skeletal neuromuscular junction. A preliminary account of this work has been reported previously (8).

Materials and Methods

Materials. DL-[7-^{3}H]-Norepinephrine ([^{3}H]NE) with a sp act of 10–15 Ci/mmol was purchased from Dupont-NEN (Boston, MA). Sigma Chemical Co. (St. Louis, MO) was the source of nicotine hydrogen tartrate, acetylcholine chloride, mecamylamine hydrochloride, decamethonium bromide, hexamethonium bromide, pentolinium tartrate, and *d*-tubocurarine

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chloride. Vinblastine sulfate was a gift from the Eli Lilly Co. (Indianapolis, IN). All other reagents, which were of analytical grade, were obtained from various commercial suppliers.

Isolation and Primary Culture of Bovine Adrenal Chromaffin Cells. Bovine adrenal glands were obtained from Herman Falter Packing Co. (Columbus, OH) and used within 30 min of their removal from the carcass. Adrenal chromaffin cells were dissociated from the intact glands, plated, and maintained at 37°C in a humidified, 5% CO_2 environment, as described previously (4, 6).

Catecholamine Secretion Studies. Secretion experiments were performed in a physiologic salt solution containing 2 m*M* Ca^{2+} and 0.5% bovine serum albumin (4). When vinblastine was used, cells were pretreated for 30 min. We have reported previously that pretreatment times between 5 min and 60 min produce the same maximum inhibition of secretion (4). The [^{3}H] NE assay, which has been described previously, was used to monitor catecholamine release from the cultured cells (4). The amount of radioactivity released during a 10-min period of stimulation was determined by liquid scintillation spectroscopy. The radioactivity remaining in the cells was then extracted with 8% trichloroacetic acid and was also counted. The sum of the secreted and the trichloroacetic acid-extractable radioactivity represented total incorporated [^{3}H]NE. Results were expressed as a percentage of the net stimulated control response where basal (nonstimulated) fractional release had been subtracted from all groups (i.e., treatment group release minus basal release divided by control acetylcholine chloride-stimulated release minus basal release × 100).

Phrenic Nerve-Hemidiaphragm Preparation. Left phrenic nerve-hemidiaphragm muscles were obtained from adult Sprague-Dawley-derived rats and suspended in 35°C Krebs medium (9) aerated with 95% oxygen and 5% carbon dioxide. A 0.5 g resting tension was imposed on the muscles and isometric contractions were recorded via a Grass force displacement transducer (F.03) and oscillograph. In some experiments, the muscles were driven by repetitive (0.5 Hz) stimulation of the phrenic nerve (square wave pulses of 1 msec duration at two times threshold voltage). Threshold voltages were 0.1–0.4 V. The basic technique followed the original design of Bülbring (10). Drug concentrations were expressed as molar bath concentrations.

Diaphragm Strips. Strips of rat diaphragm muscle approximately 4 × 15 mm were mounted in a tissue bath under conditions identical to those described for the phrenic nerve-hemidiaphragm preparation, except that all extrinsic nerves were sectioned and no electrical excitation was used to stimulate the muscle.

Statistical Treatment. Data were presented as arithmetic mean ± SE except for IC_{50} and EC_{50} values which were presented as geometric mean (95% confidence limits). In the secretion studies, *n* represents the number of observations carried out in triplicate. Sigmoid inhibition and concentration-response curves were generated by nonlinear regression (InPlot 3.1; GraphPad Software, San Diego, CA). Experimental values were compared using analysis of variance and Duncan's multiple range test (11) at a 0.05 level of significance.

Results

Mammalian Adrenal Chromaffin Cell Response. Table I summarized the inhibitor potencies (IC_{50}s) of all drugs examined. Duncan's multiple range test revealed that the drugs could be partitioned into three groups based on potency: mecamylamine = pentolinium = *d*-tubocurarine > vinblastine > hexamethonium = decamethonium. Thus, vinblastine occupied an intermediary position among these nAChR receptor antagonists. In sufficient concentration, all of the inhibitors appeared to be capable of fully reducing the release of catecholamines from cultured adrenal chromaffin cells to the basal (nonstimulated) level (Fig. 1). Vinblastine and the nicotinic receptor antagonists exerted no influence on basal catecholamine release and their inhibitory actions were specific for nicotinic receptor-stimulated release; i.e., these drugs did not inhibit release stimulated by depolarizing concentrations of potassium (data not shown). These studies demonstrate that vinblastine possesses anti-nAChR potencies in cultured adrenal chromaffin cells that are comparable to those of the more classical anti-nAChR drugs.

Mammalian Skeletal Muscle Response. To assess vinblastine's actions on skeletal muscle-type nAChR, the rat phrenic nerve-diaphragm system was examined. As seen in the oscillograph tracing of Figure 2, vinblastine (10–100 μM) failed to exert any inhibition of muscle response to phrenic nerve stimulation. However, in these concentrations, vinblastine produced a

Table I. Potencies of Inhibitors of Acetylcholine-Stimulated Catecholamine Release from Adrenal Chromaffin Cells in Culture

Inhibitor	IC_{50}[a] (μM)	95% confidence limit (μM)	Potency group[b]
Decamethonium	17.5	10.7–28.5	Rank 3
Hexamethonium	16.4	8.5–31.8	Rank 3
Vinblastine	8.9	7.1–11.1	Rank 2
d-Tubocurarine	2.21	1.35–3.61	Rank 1
Pentolinium	0.60	0.19–1.91	Rank 1
Mecamylamine	0.13	0.02–0.66	Rank 1

[a] Median inhibitory concentrations interpolated from the curves in Figure 1.
[b] Drugs at the same rank level were determined to be not significantly different in potency.

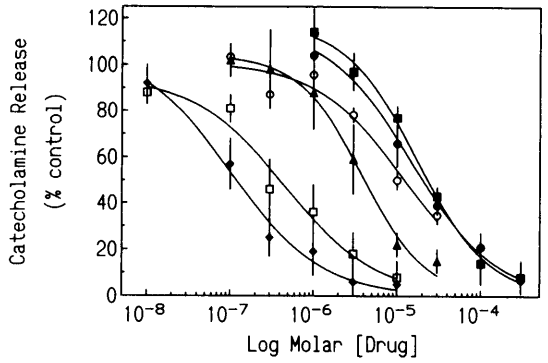

Figure 1. Log concentration-response curves of several anticholinergic drugs on acetylcholine-stimulated adrenal catecholamine release. Cultured adrenal chromaffin cells were pretreated for 30 min with various concentrations of inhibitors before stimulation with 100 μM acetylcholine in the continued presence of the drugs. Response is expressed as a percentage of the net stimulated control response. Points represent the mean ± SE (n = 4–7).

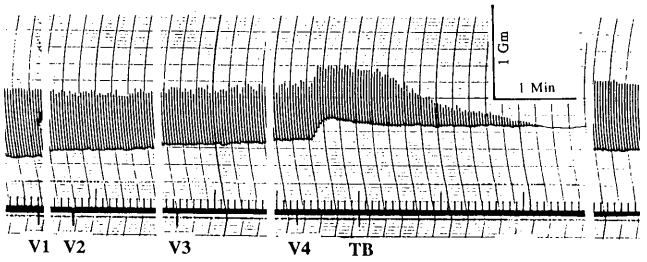

Figure 2. Response of rat hemidiaphragm preparation to left phrenic nerve stimulation. Imposed basal tension was 0.5 g. Stimulus was 0.2 V (two times threshold) at 0.5 Hz. V1 = Vinblastine, 10 μM; V2 = 20 μM; V3 = 50 μM; V4 = 100 μM; TB = d-tubocurarine, 1 μM. Concentrations of vinblastine were added in a cumulative fashion. Breaks in the oscillograph tracing represent periods during which the mechanical recording was purposely interrupted in order to compress the display. This is one of five replications.

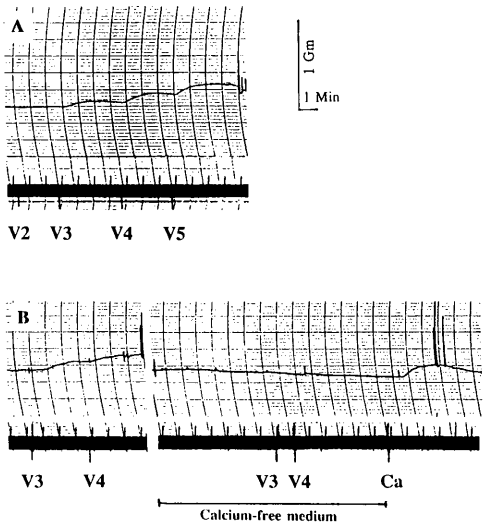

Figure 3. Response of rat diaphragm strips to vinblastine in the presence and absence of extracellular calcium. Imposed basal tension was 0.5 g. (A) Concentration-dependent change in baseline tension after cumulative doses of vinblastine in the presence of normal Krebs medium. (B) Lack of response to vinblastine in calcium-free Krebs medium and restoration of response after the addition of calcium. V2 = Vinblastine, 20 μM; V3 = 50 μM; V4 = 100 μM; V5 = 200 μM; Ca = calcium chloride, 2 mM.

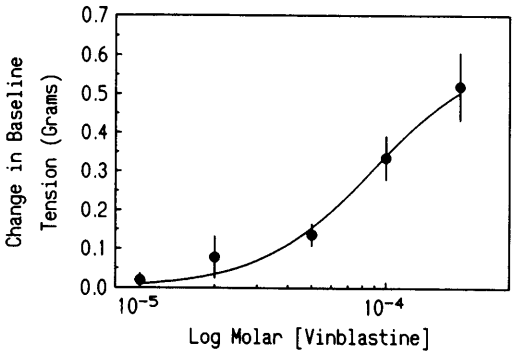

Figure 4. Log concentration-response curve of vinblastine on rat diaphragm strips. Points represent the mean ± SE of four to eight tissues. Estimated EC_{50} (95% confidence limit) = 88 (21–370) μM.

graded increase in baseline muscle tension. The rise in basal tension was accompanied by an equivalent rise in nerve-stimulated contractile peak height. Thus, there was no net change in the dimensions of the contraction spike induced by electrical excitation of the phrenic nerve. The introduction of d-tubocurarine (1 μM) gradually reduced and ultimately abolished nerve-stimulated contractions without altering vinblastine-induced elevation in basal tension. Washing the preparation with Krebs medium restored the baseline to near normal. Therefore, the vinblastine effect is considered to be reversible.

The change in basal tension that was observed with vinblastine also can be elicited in the absence of nerve stimulation as seen in Figure 3. The unstimulated diaphragm strip in Panel A (Fig. 3) exhibited a dose-dependent contraction to cumulative doses of vinblastine (20–200 μM). Replacing Krebs incubating medium with calcium-free Krebs solution prevented the vinblastine effect. The addition of calcium to the calcium-free medium restored vinblastine's ability to elevate resting tension (Panel B, Fig. 3). Although it is not depicted on the tracing, the addition of calcium to calcium-free medium in the absence of vinblastine does not produce a contracture.

The log dose-response relationship of vinblastine on passive diaphragm strips is portrayed in Figure 4. Maximum developed resting tension was estimated, by extrapolation, to be 0.61 g. The EC_{50} (95% confidence limit) was estimated to be 88 (21–370) μM.

Discussion

Nicotinic acetylcholine receptors are found in neuromuscular junctions and on neuronal and neural crest-derived tissues (e.g., sympathetic ganglia, brain, and adrenal medulla). Although skeletal muscle-type nAChR are fairly well characterized, less is known

about adrenal nAChR and other neuronal-type nAChR. Pharmacologic similarities between neuronal and muscle nAChR have been described (12, 13). Other studies, however, have indicated that these two receptor types differ in their sensitivities to various nAChR antagonists and neurotoxins (14–16). It is also becoming clear that neuronal nAChR differ from muscle nAChR in both structure and regulation (for review, see Ref. 17).

Several reports have described the anti-nAChR activity of vinblastine in neuronal tissues (2, 4, 5). We have demonstrated previously that vinblastine and other antimitotic drugs affect nAChR function in cultured bovine adrenal chromaffin cells and that these effects are noncompetitive (4, 6, 7, 18). This effect of vinblastine is specific for release stimulated via activation of adrenal nicotinic receptors; i.e., vinblastine does not inhibit release stimulated by histamine, veratridine, barium chloride, or depolarizing concentrations of potassium (4). One explanation of vinblastine's anti-nAChR activity is that vinblastine interferes with nAChR agonist binding. Trifarò *et al.* (2) have demonstrated that vinblastine (100 μM) not only inhibits catecholamine release from perfused bovine adrenal glands, but also blocks transmission through the superior cervical ganglia of the cat. They suggested that this action reflected anti-nAChR activity. McKay *et al.* (5), investigating this possibility using the nAChR-rich membrane preparation from *T. californica* electric organs, found that vinblastine did not interfere with [α-^{125}I]bungarotoxin binding to the nAChR agonist binding site. This suggests that vinblastine had little or no affinity for nAChR binding sites. However, vinblastine had other effects on binding properties to the Torpedo membranes. Vinblastine (i) interfered with binding of [^{3}H]perhydrohistrionicotoxin to the nAChR-gated ion channel and (ii) stabilized a high affinity conformation of the nAChR complex, a property it shares with many other noncompetitive inhibitors of the nAChR-gated ion channel.

In the studies reported here, we found that in adrenal chromaffin cells vinblastine has pharmacologic properties which are similar to other nAChR antagonists both in terms of potency and efficacy. Classical ganglionic blockers such as mecamylamine and pentolinium tended to be more potent than the classical skeletal myoneural blockers such as *d*-tubocurarine and decamethonium. Although nominally classified as a ganglionic blocker, hexamethonium was virtually identical in potency to that of decamethonium. Vinblastine's potency falls within the range of activities of the other inhibitors.

Vinblastine, however, exhibits no anticholinergic activity at the skeletal neuromuscular junction. Its presence, in concentrations up to 200 μM, does not impair the phrenic nerve-stimulated contraction of the innervated diaphragm muscle. One possible explanation for these negative results is that under our treatment conditions, vinblastine does not have the opportunity to reach the nicotinic receptors. However, we believe that circumstantial evidence makes it unlikely that lack of access is an explanation for the lack of inhibitory action. Under similar treatment conditions with regard to time and concentration, vinblastine has been demonstrated to exert inhibitory actions on the *in situ* superior cervical ganglia preparation (2). In addition, we have reported previously that pretreatment times of 5 min or longer produce the same maximum inhibition of adrenal secretion (4). Since total vinblastine exposure time in the diaphragm studies was 10–15 min, the lack of antinicotinic activity in rat diaphragm is probably not due to inappropriate treatment conditions.

At high doses, however, vinblastine exerted a direct concentration-dependent effect on the muscle tissue to increase resting tension, an effect which is independent of nAChR activity. The mechanism that underlies this action of vinblastine is unknown. Vinblastine has been reported to possess nonspecific membrane-related actions at high concentrations (4, 19). We do not know whether similar nonspecific membrane actions underlie vinblastine's ability to produce contracture of the diaphragm, but we have found that this effect is reversible and dependent upon extracellular calcium. Vinblastine has also been reported to possess properties similar to the noncompetitive inhibitors of the nAChR-gated ion channel such as histrionicotoxin and adiphenine (5, 11). Interestingly, colchicine, another antimitotic agent, has also been found to produce contracture of rat diaphragm (20). In the latter report, colchicine inhibited neuromuscular transmission in the isolated frog sciatic nerve-sartorius muscle preparation but *not* in the rat phrenic-nerve diaphragm preparation. These results suggest that a species dependency may further complicate attempts to discriminate among the activities of vinblastine on various nAChR.

Summary and Conclusions

1. Vinblastine possesses anti-nicotinic receptor potencies in adrenal chromaffin cells that are comparable to those of the more classical anti-nAChR drugs.

2. Vinblastine has no demonstrable anticholinergic receptor activity on rat diaphragm skeletal muscle.

3. Vinblastine produces a concentration-related elevation of basal tension in diaphragm muscle (contracture) that is independent of nicotinic receptor function. This effect requires the presence of extracellular calcium. It remains to be determined whether this effect is the result of (i) the ion channel-related properties of vinblastine, (ii) its well-characterized influences on microtubules, or (iii) some heretofore unrecognized pharmacologic attribute of vinblastine.

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