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EFFECTS OF VINCA ALKALOIDS ON UPTAKES OF AMINO ACIDS BY RAT BRAIN SYNAPTOSOMES

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Abstract—Synaptosomal uptakes of a number of amino acids were strongly inhibited by vinblastine (I_{50} = 30–50 μM). Vincristine was a less effective inhibitor and colchicine had virtually no effect. The inhibitory effects of vinblastine on uptakes of alanine, leucine and serine were modified by cations and by nucleotides. The degree of inhibition was reduced by Mg^{2+}, Ca^{2+} and Na^{+}; nucleotides antagonized the inhibitory effect of vinblastine but this effect required the presence of divalent cations. The inhibitory effects of vinblastine were almost abolished when the incubations were carried out at 5° instead of 37°. The efflux of amino acids from synaptosomal particles was retarded by vinblastine; this effect could be observed at a concentration as low as 15 μM. By analogy with the effects of vinca alkaloids on biological processes in other tissues, our present results suggest that neurotubular protein participates in the initial rapid uptake and the subsequent efflux of amino acids in synaptosomal fractions.

Amino acid transport systems which are present in isolated nerve endings have been a subject of primary interest in this laboratory [1–3]. Although the relationship of these transport systems to synaptic function is unclear, one potential importance of these systems stems from the fact that some amino acids serve as precursors in the biosynthesis of neurotransmitters. Furthermore, recent studies [4,5] indicate that certain other amino acids may themselves act as neurotransmitter substances.

There is a growing amount of evidence [6–8] that the release of some neurotransmitters at the synaptic junction, like many similar stimulus-release processes in other tissues (e.g. insulin secretion [9]), is mediated by a contractile mechanism involving an actomyosin-like protein. The effects that vinca alkaloids and colchicine have on neurotransmitter transport processes in isolated synaptosomal systems [6] strongly suggest that these mechanisms are associated with a microtubular system. In the present investigation, we have studied the effects of vinca alkaloids on synaptosomal transport of amino acids. It is shown that the characteristics of vinblastine inhibition of the transport of a number of amino acids are similar to those reported [6] for synaptosomal transport of several neurotransmitter substances, and the results raise the possibility that a microtubular system mediates the synaptosomal uptakes of these amino acids in much the same manner as those of the neurotransmitters.

MATERIALS AND METHODS

Materials

Vinblastine sulfate and vincristine sulfate were gifts from Eli Lilly & Co. (Indianapolis, Ind.). Colchicine was obtained from Sigma Chemical Co. (St. Louis, Mo.). Uniformly labeled L-[^{14}C]-amino acids were purchased from New England Nuclear Corp. (Boston, Mass.) and had the following specific radioactivities (mCi/m-mole): alanine, 139; aspartic acid, 208; glutamic acid, 240; glycine, 104; histidine, 310; isoleucine, 298; leucine, 312; phenylalanine, 459; proline, 230; serine, 155; threonine, 205; and valine, 210. Uniformly labeled L-[^{3}H]tyrosine was also purchased from New England Nuclear Corp. and had a specific radioactivity of 60 Ci/m-mole.

Assay of synaptosomal amino acid uptake

Cerebral cortices obtained from adult Sprague–Dawley rats were homogenized in 10 vol. of ice-cold 0.32 M sucrose solution. Synaptosomal fractions were prepared from the homogenates by the method of Kurokawa *et al.* [10] and suspended in one of the following five media: (1) medium TS containing 10 mM Tris–HCl (pH 7.4) and 345 mM sucrose, (2) medium TMS containing 10 mM Tris–HCl (pH 7.4), 15 mM $MgCl_2$ and 300 mM sucrose, (3) medium TCS containing 10 mM Tris–HCl (pH 7.4), 15 mM $CaCl_2$ and 300 mM sucrose, (4) medium TNS containing 10 mM Tris–HCl (pH 7.4), 22.5 mM NaCl and 300 mM sucrose, and (5) medium TMNa containing 10 mM Tris–HCl (pH 7.4), 15 mM $MgCl_2$ and 150 mM NaCl. A 1-ml portion of each suspension (containing 0.1 to 0.2 mg of synaptosomal protein) was incubated with a labeled amino acid (0.1 μCi) and various concentrations of inhibitors and nucleotides under the conditions given in the legends to the tables and figures. At the end of the incubation period the particles were collected on Millipore filters and assayed for radioactivity as described elsewhere [1].

RESULTS

Characteristics of vinblastine inhibition of amino acid uptake by synaptosomal fractions

The inhibitory effects of the vinca compounds have been expressed in terms of the I_{50} concentration (the concentration of inhibitor that produced a 50 per cent inhibition in synaptosomal uptake of substrate amino acids). The values for I_{50} were derived from Hill-type

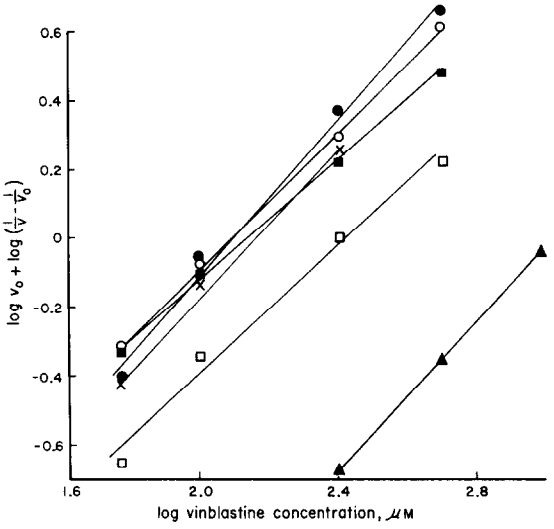

Fig. 1. Vinblastine inhibition of amino acid uptake by synaptosomal fractions prepared from rat cerebral cortex. Synaptosomal fractions prepared as described in Methods were incubated with 0.1 μCi (0.2 to 0.8 μM) of the labeled amino acid and the various concentrations of vinblastine sulfate, at 37° for 1 min. For proline uptake the incubation medium was TMNa and for the other amino acids the incubation medium was TMS. Key: v_0 is the rate of uninhibited amino acid accumulation and v the rate of accumulation in the presence of vinblastine; ●——●, serine; ○——○, leucine; ■——■, alanine; □——□, glycine; ▲——▲, proline; and ×——×, leucine accumulation when the leucine concentration in the incubation mixtures was 10 μM.

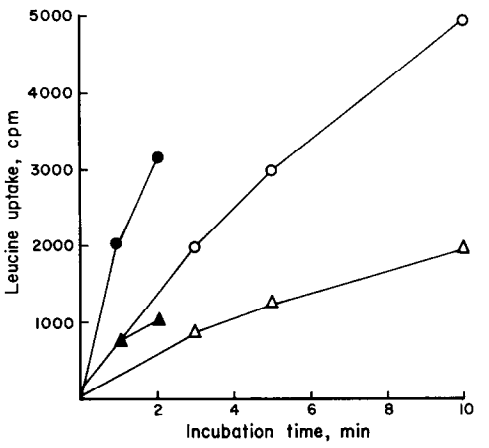

Fig. 2. Effects of vinblastine on the initial rate of leucine uptake. Synaptosomal fractions were incubated with 0.1 μCi of labeled leucine for various time intervals. The incubation medium was TMS. Key: ●——●, incubation at 37° in the absence of vinblastine; ▲——▲, incubation at 37° in the presence of 250 μM vinblastine; ○——○, incubation at 17° in the absence of vinblastine; △——△, incubation at 17° in the presence of 250 μM vinblastine.

plots, in which $\log[(v_0 - v)/v]$ (v_0 is the uptake rate in the absence of inhibitor and v the rate in the presence of inhibitor at a concentration, [I]) was plotted against log [I]. Each plot was constructed from data obtained with three to four concentrations of the inhibitor substance. The plots were approximately linear in all cases, and their regression equations were computed by the method of least squares. The I_{50} values are the inhibitor concentrations at which the values for $\log[(v_0 - v)/v]$ are zero and were calculated from the regression equations. Representative Hill plots of vinblastine inhibition are shown in Fig. 1. The slope of each plot is approximately unity, suggesting a first-order interaction of vinblastine with each of the amino acid transport processes [11].

Data presented in Table 1 compare the inhibitory effects of vinblastine on the accumulations of nine different amino acids. Vinblastine strongly inhibited the uptakes of leucine, isoleucine, valine, histidine, tyrosine, phenylalanine, serine and alanine, with I_{50}

Table 1. Inhibition of synaptosomal uptake of amino acids by vinblastine*

Amino acid†	I_{50} (μM)
Alanine	137 ± 10 (7)
Glycine	313 ± 62 (3)
Histidine	108 ± 38 (3)
Isoleucine	107 ± 28 (3)
Leucine	105 ± 7 (8)
Phenylalanine	115 ± 36 (3)
Serine	93 ± 10 (6)
Tyrosine	63 ± 10 (3)
Valine	91 ± 21 (3)

* Synaptosomal fractions prepared as described in Methods were incubated with the labeled amino acids for 1 min at 37° in a final volume of 1 ml of medium TMS. The incubation mixtures contained various concentrations of vinblastine sulfate. I_{50} values (given with the standard deviations) were determined from Hill plots, as described in Methods. The numbers in parentheses are the numbers of experiments.

† All of the amino acids except tyrosine were labeled uniformly with [^{14}C]; tyrosine was labeled with tritium.

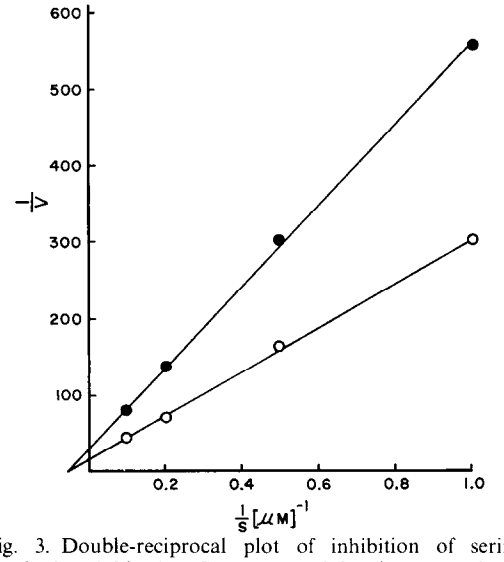

Fig. 3. Double-reciprocal plot of inhibition of serine uptake by vinblastine. Synaptosomal fractions were incubated with 0.1 μCi of labeled serine and various concentrations of unlabeled serine at 37° for 1 min. The incubation medium was TMS. Key: ○——○, serine uptake in the absence of vinblastine; ●——●, serine uptake in the presence of 100 μM vinblastine.

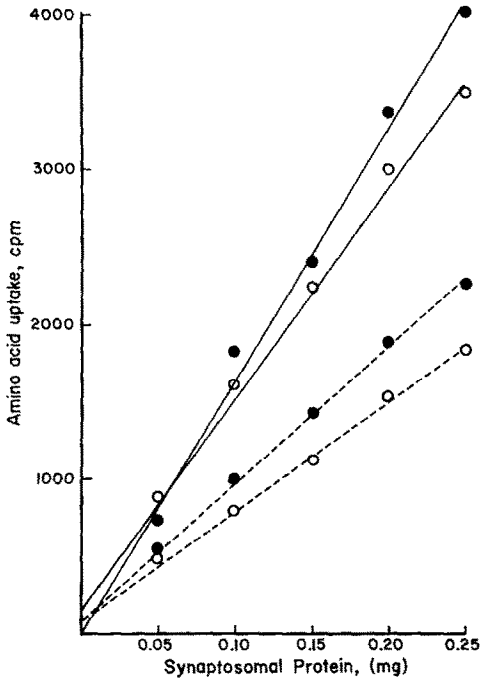

Fig. 4. Effects of vinblastine on serine accumulation as a function of synaptosomal protein concentration. Various concentrations of synaptosomal protein were incubated with 0.1 μCi of labeled amino acid at 37° for 1 min. The incubation medium was TMS. Key: ●——●, accumulation of serine in the absence of vinblastine; ●- - -●, accumulation of serine in the presence of 100 μM vinblastine; O——O, accumulation of leucine in the absence of vinblastine; O- - -O, accumulation of leucine in the presence of 100 μM vinblastine.

values in the range of 100 μM. The alkaloid had a considerably lesser inhibitory effect on the uptake of glycine, as indicated by the 3-fold higher value for I_{50}.

The effects of vinblastine on the initial rate of leucine uptake at two incubation temperatures are shown in Fig. 2. The early influx of leucine was inhibited more at 37° than at 17°, though at both the temperatures the extent of inhibition remained constant during the entire time interval studied.

Experiments in which the concentrations of substrate amino acids were varied indicated that vinblastine inhibition of synaptosomal amino acid uptake was of the noncompetitive type. Noncompetitive inhibition of serine uptake is demonstrated by the double-reciprocal plot in Fig. 3. Noncompetitive inhibition of leucine uptake can be inferred from the fact that the velocity function of the Hill plot (ordinate in Fig. 1) remained unchanged when the concentration of leucine was increased to 10 μM (see Fig. 1). Under these conditions, the I_{50} value is independent of the substrate concentration and is equivalent to the inhibitory constant, K_i, derived from Michaelis-Menten kinetics (see Ref. 11).

The extent of vinblastine inhibition was not dependent on the concentration of synaptosomal protein in the incubation mixture. A plot of amino acid uptake against protein concentration (Fig. 4) resulted in a straight line through the origin in the presence as well as in the absence of inhibitor, and hence the inhibition conforms to a reversible type [12].

Factors which influence vinblastine inhibition of amino acid uptake

A number of factors were found to alter the effectiveness of vinblastine as an inhibitor of synaptosomal amino acid uptake. The quantitative effects of these factors were measured as changes in the I_{50} values for vinblastine inhibition.

Temperature. The inhibition of amino acid uptake by vinblastine occurred to a much lesser extent at 5° than at 37° (Table 2). This effect of reduced temperature was most pronounced in the case of alanine uptake, where the I_{50} value at 5° was about ten times the value determined at 37°. The I_{50} values for serine and leucine accumulations were about four times greater at 5° than at 37°.

Cations. The above experiments were carried out with incubation media which contained 15 mM Mg^{2+}. The initial rate of amino acid accumulation from medium lacking divalent cations is only about $\frac{1}{2}$ of that from medium containing either Mg^{2+} or Ca^{2+} (unpublished data). However, vinblastine had a substantially greater inhibitory effect in cation-free medium than in medium containing either of the divalent cations (Table 3). This effect was more pronounced in the cases of serine and leucine uptakes, where the I_{50} values for vinblastine inhibition were 2.5- to 5-fold less in TS medium. Additional experiments showed that the uptakes of valine, isoleucine, histidine, phenylalanine, tyrosine and threonine from TS media were also more strongly inhibited by vinblastine than the corresponding uptakes from TMS or TCS medium. The data given in Table 3 further show that Na^+ acts in a manner similar to the divalent cations in reducing the vinblastine inhibition of the uptakes of the three amino acids. These experiments

Table 2. Effect of incubation temperature on vinblastine inhibition of amino acid accumulations by synaptosomal fractions*

Incubation temperature	I_{50} (μM)		
	Leucine	Serine	Alanine
37°	105 ± 7 (8)	93 ± 10 (6)	137 ± 10 (7)
5°	407 ± 44 (6)	370 ± 32 (5)	> 1000 (5)

* Synaptosomal fractions were incubated with labeled amino acids in medium TMS at 37° for 1 min or at 5° for 10 min. Vinblastine was included at various concentrations in the incubation mixtures. I_{50} values were computed as described in the text.

Table 3. Effects of cations alone, and in combination with ATP, on vinblastine inhibition of synaptosomal amino acid uptake*

Amino acids	Additions (0.5 mM)	I_{50} values for vinblastine inhibition of synaptosomal amino acid uptake from medium			
		TS	TMS	TCS	TNS
Serine	None	30 ± 7 (11)	93 ± 10 (6)	172 ± 30 (4)	128 ± 13 (3)
	ATP	65 ± 12 (4)	345 ± 47 (5)	653 ± 146 (3)	181 ± 21 (3)
Leucine	None	42 ± 3 (3)	105 ± 7 (8)	143 ± 22 (5)	180 ± 20 (3)
	ATP	53 ± 15 (3)	720 ± 80 (5)	1040 ± 146 (3)	325 ± 33 (3)
Alanine	None	86 ± 20 (4)	137 ± 10 (7)	244 ± 41 (4)	136 ± 4 (2)
	ATP	115 ± 12 (3)	665 ± 90 (3)	570 ± 90 (3)	224 ± 13 (3)

* Synaptosomal fractions were incubated with the labeled amino acids for 1 min at 37° in the incubation media indicated. The I_{50} values for vinblastine inhibition of the amino acid uptake were computed as described in the text.

were concerned with amino acids which are maximally accumulated by synaptosomal fractions in the absence of Na^+ [1]. Initial experiments with aspartic acid, which is transported via a totally Na^+-dependent system, indicated a virtual lack of vinblastine inhibition in the presence of 150 mM Na^+. Since it was not clear whether Na^+-dependent uptake mechanisms *per se* might be insensitive to vinblastine, or whether any possible inhibitory effects were masked by the high Na^+ concentration, the effects of vinblastine on Na^+-dependent systems were studied at suboptimal Na^+ concentrations. Data for aspartic acid and glutamic acid, summarized in Table 4, show that the effects of vinblastine were considerably modified by Na^+. At the lowest Na^+ concentration, 1 mM, vinblastine did in fact substantially inhibit the uptakes of both amino acids. The alkaloid had progressively less effect on the uptakes at increasing Na^+ concentrations, and, in the case of aspartic acid, had virtually no effect when the Na^+ concentration was 150 mM. The data in Table 4 also show an interesting effect of Na^+ on the slope of the Hill plot. The slope is unity or slightly greater than unity at a Na^+ concentration of 1 mM and increases with increasing Na^+ concentration, reaching values greater than 2 at the higher Na^+ concentration. A possible interpretation of this change in the slope value is that increasing Na^+ concentration results in an increase in the number of vinblastine molecules which interact at the transport site (see Ref. 11). This effect of Na^+ would be in addition to its effect in antagonizing the vinblastine inhibition of the uptake rate, as indicated by the increase in the I_{50} value. Essentially similar results were obtained in experiments with proline, another amino acid which is accumulated by synaptosomes via a totally Na^+-dependent system [1].

Nucleotides. When divalent cations were present in the medium, the inhibitory effects of vinblastine were antagonized by a number of nucleotides. Among the nucleotides studied, ATP caused the greatest reduction in the vinblastine inhibition. In the presence of ATP, and in medium TMS or TCS, the I_{50} value for vinblastine inhibition of serine uptake was increased by 3-fold and the values for leucine and alanine uptakes by nearly 7-fold (Table 3). cAMP and GTP increased the I_{50} values by 2- to 3-fold. The following nucleotides produced a relatively small but statistically significant ($P < 0.01$) increase in the I_{50} value for vinblastine inhibition of serine uptake from medium TMS: ITP, UTP, CTP, and β,γ-methylene ATP. Adenosine and β,γ-methylene GTP had no effect.

It can be seen from the data in Table 3 that the ability of ATP to antagonize the inhibitory effects of

Table 4. Effects of Na^+ on the slope of the Hill plot and I_{50} for vinblastine inhibition of synaptosomal aspartic acid and glutamic acid uptakes*

Na^+ concn (mM)	I_{50}		Slope	
	Aspartic acid	Glutamic acid	Aspartic acid	Glutamic acid
1	166 ± 17 (6)	188 ± 62 (4)	1.25 ± 0.17 (6)	1.07 ± 0.31 (4)
2	172 ± 10 (2)	156 ± 2 (2)	1.63 ± 0.05 (2)	1.33 ± 0.08 (2)
5	213 ± 24 (3)	188 ± 27 (5)	1.67 ± 0.28 (3)	1.53 ± 0.16 (5)
10	390 ± 25 (4)	290 ± 12 (4)	2.28 ± 0.12 (4)	1.57 ± 0.23 (4)
20	610 ± 70 (3)	340 ± 40 (4)	2.55 ± 0.17 (3)	1.66 ± 0.13 (4)
50	1000 ± 10 (2)	580 ± 40 (3)	2.41 ± 0.40 (2)	2.59 ± 0.34 (3)
150	≫ 1000† (3)	590 ± 20 (2)		2.65 ± 0.25 (2)

* Synaptosomal fractions were incubated with either [^{14}C]aspartic acid or [^{14}C]glutamic acid for 1 min at 37°. The incubation medium contained various concentrations of Na^+ and vinblastine. Hill plots of the vinblastine inhibition were constructed and the slopes and I_{50} values were derived from the latter plots. Numbers in parentheses represent the number of determinations.

† Vinblastine at a concentration of 1000 μM caused 12 per cent inhibition of uptake.

Table 5. Effects of vincristine on synaptosomal amino acid uptake*

Amino acid	I_{50} (μM) Medium TS	Medium TMS
Serine	180 ± 20 (2)	1000 ± 400 (3)
Leucine	430 ± 110 (2)	1000 ± 300 (3)

* Synaptosomal fractions were incubated with the labeled amino acid for 1 min at 37° in either medium TS or medium TMS.

vinblastine is largely dependent on the presence of divalent cations; in both TS and TNS media ATP had little or no effect.

Effects of vincristine and colchicine on synaptosomal amino acid uptake

A comparison was made of the inhibitory effects of vinblastine with another vinca alkaloid, viz. vincristine, and with colchicine. As in the case of vinblastine the inhibitory effects of vincristine on serine and leucine uptakes were substantially greater in cation-free medium than in medium containing Mg^{2+} (Table 5). However, quantitatively, vincristine exerted a much lesser inhibition than vinblastine. The I_{50} values for vincristine inhibition of serine, leucine and alanine were 6- to 10-fold higher than those for inhibition by vinblastine. Colchicine, at comparable concentrations, produced practically no inhibition of amino acid uptake. Even at a concentration of 1 mM, only 20 per cent inhibition could be observed with colchicine.

Effects of vinblastine on efflux of amino acids

The initial rapid influx of amino acids into synaptosomal fractions is followed by a period of progressive efflux [1]. The effects of vinblastine on this efflux process were studied.

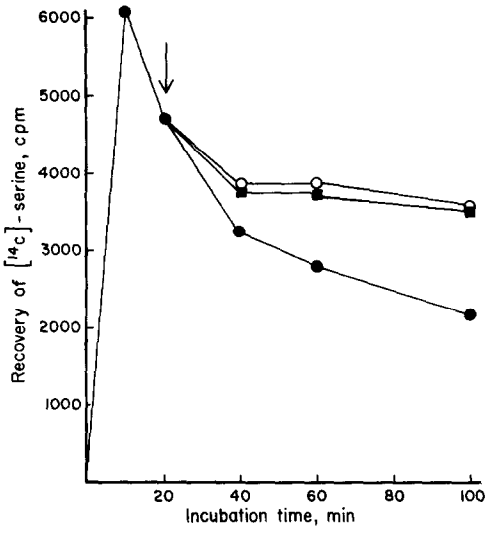

Fig. 6. Recovery of [^{14}C]serine from synaptosomal particles as a function of incubation time. Synaptosomal fractions were incubated with 0.1 μCi of labeled serine in medium TS for time intervals ranging from 3 to 90 min. After 20 min of incubation (indicated by arrow) vinblastine was added to final concentrations of 30 and 60 μM. Key: ●—●, incubation mixtures containing no vinblastine; ○—○, incubation mixtures containing vinblastine at a final concentration of 30 μM; ■—■, incubation mixtures containing vinblastine at a final concentration of 60 μM.

In the first type of experiment, vinblastine was added to the incubation mixtures at the beginning of the incubation period. In the absence of vinblastine, the net loss of amino acid began after about 10 min. With vinblastine in the medium, efflux did not begin until after about 30 min of incubation, and thereafter proceeded at a relatively slow rate. Figure 5 shows that vinblastine, at concentrations of 15 and 30 μM, prolonged the retention of the amino acid over a period of 90 min.

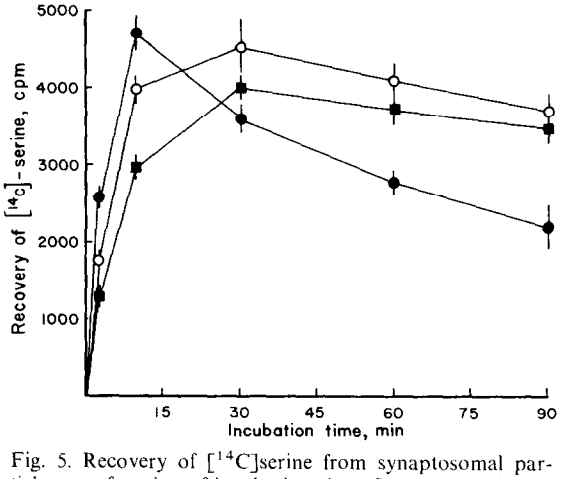

Fig. 5. Recovery of [^{14}C]serine from synaptosomal particles as a function of incubation time. Synaptosomal fractions were incubated with 0.1 μCi of labeled serine in medium TS for time intervals ranging from 3 to 90 min. Key: ○—○, incubation in the presence of 15 μM vinblastine; ■—■, incubation in the presence of 30 μM vinblastine; ●—●, incubation in the absence of vinblastine.

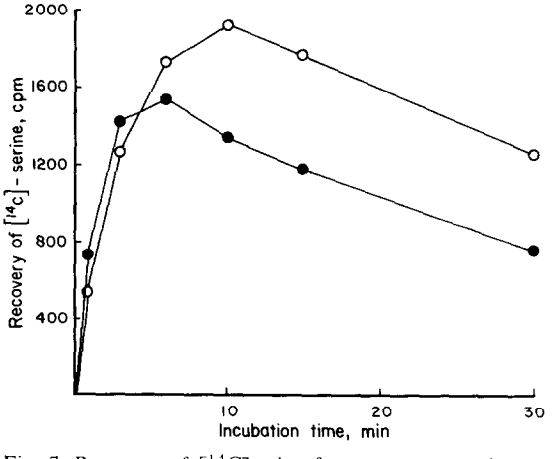

Fig. 7. Recovery of [^{14}C]serine from synaptosomal particles as a function of incubation time. Synaptosomal particles were incubated with 0.1 μCi of labeled serine in medium TMNa. Key: ●—●, amino acid recovery in the absence of vinblastine; ○—○, amino acid recovery in the presence of 60 μM vinblastine.

In the second type of experiment, vinblastine was added to the incubation mixtures after 20 min of incubation. As can be seen in Fig. 6, the fractions progressively lost amino acid in the absence of vinblastine, but in the presence of vinblastine, retained a relatively constant amount of amino acid over a 60-min period.

These experiments on the effects of vinblastine on amino acid efflux were carried out in TS medium. In media containing Na^+ and Mg^{2+} (medium TMNa), the efflux of amino acid was substantially enhanced. Vinblastine still clearly reduced the efflux of amino acid from this latter medium (Fig. 7).

DISCUSSION

The magnitude of vinblastine inhibition of the synaptosomal amino acid transport systems studied in the present investigation is comparable to that reported by Nicklas *et al.* [6] for inhibition of synaptosomal uptake of DOPA, norepinephrine and γ-aminobutyric acid. In contrast to vinblastine, vincristine was a weak inhibitor of synaptosomal amino acid uptake. The study of Secret *et al.* [13] on the binding of vinca alkaloids by rat blood platelets is pertinent to our results. In the platelet system, vincristine interacted much more slowly with platelet-binding sites than did vinblastine, in terms of its ability to displace vinblastine. The authors give two possible interpretations of this finding. The first, which they favor, is that vincristine shares the same binding site as vinblastine, but binds to the site more weakly than vinblastine; the second is that vincristine binds to a different site but modifies the binding characteristics of the vinblastine site. In either case, we consider it possible that an analogous dissimilarity in the interaction of the two alkaloids with synaptosomal amino acid transport sites could account for the difference in the degree of inhibition of amino acid uptake.

It should be noted that the more neurotoxic of these two vinca alkaloids, vincristine, is the less effective inhibitor of synaptosomal amino acid uptake. Since there is apparently little difference in the degree to which the two compounds gain entry into the CNS, a correlation cannot be made between their neurotoxic effects and their inhibitory effects on synaptosomal transport of amino acids.

Colchicine had practically no effect on synaptosomal amino acid accumulation; even at a concentration of 1 mM, it produced only a slight inhibition. Several investigators have observed such a dissimilarity in the inhibitory effects of vinca alkaloids and colchicine in systems known or presumed to be mediated by microtubules. Thoa *et al.* [14], for example, found vinblastine to be about ten times more effective than colchicine in blocking the phenoxybenzamine-stimulated release of norepinephrine and of dopamine-β-hydroxylase from sympathetic nerves. In the experiments described by Poisner and Bernstein [15], vinblastine was about ten times more effective in inhibiting catecholamine release from the adrenal medulla than was colchicine. Moreover, colchicine had relatively little effect, as compared to vinblastine, on the synaptosomal uptakes of putative transmitter substances [6]. There has been disparity in the reported effects of colchicine on fast axonal transport. Some investigators have observed colchicine to be as inhibitory as vinblastine, whereas others found little or no colchicine inhibition. Although the lack of a colchicine effect questions an independent, role for a neurotubular system in axonal transport, Redburn and Cotman [16] suggest that maximum sensitivity of the transport system to colchicine may require a prolonged exposure to the drug. In fact, Wilson [17] has shown that colchicine complexes with isolated chick brain tubulin protein at a very slow rate; this binding process does not attain equilibrium for several hr at 37°. An insufficient exposure time could, therefore, account for our failure to observe colchicine inhibition. We could not ascertain if colchicine could inhibit synaptosomal amino acid transport after a long duration of exposure, because the transport systems are labile and display net uptake of amino acid only during the first few min of incubation.

The inhibitory effects of vinblastine on amino acid uptake were almost abolished when the incubations were carried out at 5° instead of at 37°. A reduction in temperature causes characteristic changes in the conformation of microtubular protein [18]. Such conformational changes could also alter vinblastine binding and thereby reduce its inhibitory effects on amino acid uptake. Consistent with this possibility is the fact that vinblastine binding to rat blood platelets is nearly 5-fold less at 4° than at 37° [13].

The inhibitory effects of vinblastine were greatest in incubation media solely containing Tris–HCl buffer and sucrose. The inclusion of Na^+ in the incubation media substantially increased the I_{50} values for vinblastine inhibition; this effect was observed in the case of both amino acids which do not require Na^+ for uptake (leucine, serine, alanine) and those which are transported via strictly Na^+-dependent systems (aspartic acid, glutamic acid, proline). Nicklas *et al.* [6] have reported a relatively weak inhibitory effect of vinblastine on glutamic acid uptake by synaptosomal preparations. Our data indicate that the lack of pronounced inhibition of glutamic acid uptake by vinblastine, reported by Nicklas *et al.* [6], is perhaps a result of the high Na^+ levels employed in their studies. Under the experimental conditions used by these authors (high Na^+), the synaptosomal uptake of γ-aminobutyric acid was strongly inhibited by low concentrations ($\sim 50\ \mu M$) of vinblastine. A strong inhibition of γ-aminobutyric acid uptake by vinblastine occurred under our experimental conditions as well; moreover, we found that this inhibitory effect was not changed by a reduction in Na^+. The latter observation is consistent with the suggestion that the sites of glutamate and γ-aminobutyric acid accumulations are distinct [6].

The inhibitory effects of vinblastine on synaptosomal amino acid uptakes were reduced by Mg^{2+} and Ca^{2+}. Nucleotides antagonized vinblastine inhibition, but this effect required the presence of either Ca^{2+} or Mg^{2+}. The ionic factors and nucleotides which modify vinblastine inhibition of synaptosomal amino acid uptake all presumably have a fundamental involvement with the structure and function of tubular protein and microtubular systems [19]. Evidence in support of such an involvement is derived in part from the stabilizing effects which these agents have

in terms of colchicine-binding activity of microtubular protein. Colchicine-binding activity is stabilized by high ionic strength medium, by Mg^{2+}, and by GTP in the presence of Mg^{2+}. This stabilizing action has been attributed to conformational changes in the tubulin protein which retard the release of bound colchicine. Moreover, there is much evidence which indicates that vinblastine (also a stabilizer of colchicine-binding activity), guanine nucleotides and colchicine all bind to microtubular protein at different sites. Ca^{2+}, as well as Mg^{2+}, may play a role in microtubular assembly, and the possibility has been raised that tubulin possesses a high affinity binding site for Ca^{2+} which is related to the vinblastine-binding site [19].

There were quantitative differences in the effects which the nucleotides had on the uptakes of leucine, serine and alanine. ATP caused a much greater reduction in the vinblastine inhibition of leucine and alanine uptake than in vinblastine inhibition of serine uptake. We have also observed that cAMP had a greater effect in reducing vinblastine inhibition of alanine uptake than of either serine or leucine uptake (data not shown). These quantitative differences would seem to indicate that the three amino acids are transported by dissimilar mechanisms at independent sites. Further evidence for an independent alanine system can be seen in the far greater effect of reduced temperatures on the synaptosomal uptake of this amino acid.

Amino acid that is accumulated by synaptosomal particles is extruded from the particles after a short period of incubation. This efflux is retarded by vinblastine at a concentration as low as 15 μM (Figs. 5 and 6). Previous studies [1] suggested that the retention of amino acid is dependent in part on the activity of Na^+-K^+-dependent ATPase, and that efflux is coupled in some manner with ionic movements. Thus, it was observed that: (1) the efflux was greater (sooner onset and more rapid rate) from medium containing 150 mM Na^+ than from Tris–sucrose medium; (2) the addition of K^+ substantially reduced amino acid efflux in the Na^+-containing medium; and (3) K^+ had no such effect in the presence of ouabain. Synaptosomal fractions are known to be capable of exchanging Na^+ and K^+. In the Na^+-containing medium, they would presumably fill up with Na^+ and, by ion exchange, lose internal K^+. The resulting depletion of K^+ would in turn reduce the ATPase activity. The pronounced effect which vinblastine had in promoting the retention of amino acid in the Na^+-containing medium (Fig. 7) strongly suggests that this efflux of amino acid involves an active role of microtubular protein.

The work of Nicklas *et al.* [6] indicates that actin-like protein is involved in the uptake and release of putative neurotransmitters by synaptosomes. Actin-like protein as well as tubulin protein has been isolated from synaptosomal fractions [20] and from growing nerve cells [21], and both of these protein systems have recently been shown to interact with vinca alkaloids [21]. Vinblastine sulfate can precipitate a variety of cell structure proteins much in the same manner as tubulin and actin [22], and we cannot rule out possible roles for such proteins in the synaptosomal transport processes. However, the strong similarities between the effects that these alkaloids have on synaptosomal amino acid uptake and the effects observed by Nicklas *et al.* [6] on neurotransmitter uptake suggest a role for actin-like protein in synaptosomal amino acid uptake also. Since actin and tubulin are major constituents of the synaptic membrane [8], it may well be that either each of these proteins alone or both together are involved in amino acid uptake and release processes in nerve endings.

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