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Inhibition of High Affinity Synaptosomal Uptake Systems by Verapamil

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SUMMARY

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Verapamil and its analogue D-600 have been found to inhibit the uptake of serotonin, dopamine, norepinephrine and choline into rat forebrain synaptosomes. The concentrations of verapamil required for 50% inhibition of the four uptake systems were 2.3 μM, 18 μM, 32 μM and 150 μM, respectively. The inhibitory effect of verapamil on serotonin uptake was studied in detail and found to have a very rapid (less than 1 min) time of onset and an almost equally rapid rate of reversal. Inhibition appeared to be non-competitive with respect to substrate concentration. The inhibitory effects of verapamil did not seem to be due to its ability to block Ca^{++} movements since serotonin uptake was not dependent on Ca^{++} but actually inhibited by elevated Ca^{++}, and the inhibition of uptake by verapamil could not be reversed by elevation of Ca^{++}. However, due to an increased potency of verapamil at lowered Na^+ concentrations, the Na^+-dependency of the uptake processes, and verapamil's known effects on "slow" Na^+ currents, we propose that verapamil may be interfering with a Na^+-dependent component of the uptake systems.

INTRODUCTION

In recent years, verapamil has become an increasingly important agent both as a tool for studying movements of Ca^{++} and as a potentially useful therapeutic agent for treatment of certain cardiac arrhythmias (1). Evidence also has been gathered suggesting that it may be useful for the treatment of hypertension (2).

The actions of VP[1] have been studied extensively in cardiac tissue because it is in this tissue that its physiological effects seem most important. The primary observed effect of VP is on the cardiac action potential; the drug has little effect on the fast depolarization phase of the action potential (tetrodotoxin-sensitive Na^+ conductance) but blocks the prolonged depolarization phase (3, 4), which generally has been thought to be carried by Ca^{++} ions. Thus, the conclusion was reached that the actions of verapamil are due to a rather specific blockade of "slow" Ca^{++} channels (1). This blockade of Ca^{++} channels also has been suggested as the explanation for the ability of VP to block vascular smooth muscle contraction, thus causing peripheral and coronary vasodilation (5). In addition to its action of blocking slow Ca^{++} currents, Shigenobu *et al.* (6, 7) have shown that VP can block "slow" Na^+ currents. These slow Na^+ currents (distinct from tetrodotoxin-sensitive action potential currents) are generally small under most conditions but they can be easily observed in certain systems (6, 7).

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[1] The abbreviations are: VP, verapamil; KRP, Krebs-Ringer phosphate buffer.

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The experiments reported here describe another effect of VP and its analogue, D-600: the ability to interfere with several different high affinity synaptosomal uptake systems. A preliminary report of some of this work has appeared (8).

METHODS

Synaptosomes were isolated (9) from male Sprague-Dawley rats (200–300 g) by homogenization of whole forebrains in ice cold 0.32 M sucrose (8 ml/gm wet weight) using a motor driven Potter-Elvehjm homogenizer (8–10 strokes, approximately 2000 rpm). The homogenate was centrifuged for 10 min at 800 × *g*, the pellet discarded and then synaptosomes isolated by centrifugation at 8,000 × *g* for 10 min (supernatant discarded). Synaptosomes were then resuspended in 0.32 M sucrose and kept on ice until being used for uptake measurements (no more than 30 min between the end of synaptosome isolation and the beginning of the uptake experiments). Although the homogenate fraction being studied will be referred to as the synaptosomal fraction it really represents a crude fraction containing mitochondria and some other structures in addition to synaptosomes.

The uptake of radioactive substrates was measured by incubating synaptosomes in KRP which contained the following: NaCl, 126 mM; Na_2HPO_4, 15.8 mM; glucose, 10 mM; KCl, 4.75 mM; $MgSO_4$, 1.42 mM; $CaCl_2$, 1.27 mM; pargyline, 100 μM; ascorbic acid, 1 mM; pH adjusted to 7.40, osmolarity 320 mOsm/L. In experiments in which ion substitutions were made, NaCl and sucrose were added or deleted as required to maintain the osmolarity at 320 mOsm/L. In experiments studying the effects of elevated Ca^{++}, 15.8 mM Tris·Cl was substituted for Na_2HPO_4 to avoid precipitation of calcium phosphate. Likewise, when studying the effects of decreasing the concentration of NaCl, Tris·PO_4 was substituted for Na_2HPO_4 to allow measurements to be made at very low NaCl concentrations. Except where noted for individual experiments the measurement of synaptosomal uptake was made by adding 50 μl of synaptosomes (equivalent to 6.25 mg tissue) to 400 μl of KRP (pre-warmed to 37° and containing VP or D-600 as indicated) in 12 mm × 75 mm polypropylene tubes. After allowing 4 min for equilibration, uptake was initiated by the addition of 50 μl KRP containing ^{3}H-serotonin, ^{3}H-dopamine, ^{3}H-norepinephrine or ^{3}H-choline (New England Nuclear Corporation, 2–10 Ci/mmole, final substrate concentration 40 nM). Uptake was allowed to proceed for 4 min and then terminated by transferring the tubes to an ice bath and adding 3 ml ice cold KRP. Synaptosomes were then isolated by centrifugation at 6,000 × *g* for 10 min, the supernatant was discarded, and the synaptosomes washed by resuspension in 2 ml cold KRP and centrifugation at 6,000 × *g* for 10 min. Additional washing of the synaptosomes caused little if any decrease in apparent uptake. The synaptosomal pellet was dissolved directly in 7 ml Scintiverse liquid scintillation cocktail (Fisher) and the amount of radioactivity determined in glass minivials (Research Products International). Initial experiments demonstrated that more elaborate solubilization procedures were not necessary for accurate determination of the ^{3}H content of synaptosomes. Diffusional uptake and binding of ^{3}H-compounds (10% or less of total uptake) was corrected for by subtraction of the amount of uptake observed during incubations at 0° from that observed at 37°. In general, each assay condition was performed in quadruplicate and each experimental protocol repeated using two or three different synaptosomal preparations.

Using the assay conditions described above the uptake of choline, norepinephrine and dopamine by synaptosomes was linear for at least 6 min. However, the rate of serotonin uptake was not linear during this time, being somewhat greater during the first 1 min than during subsequent times. Several different modifications of the standard assay conditions, including increasing the assay volume to 1.0 ml, decreasing the temperature to 30° and changing the concentration of serotonin in the assay, did not change this apparent non-linearity of serotonin uptake. In order to examine the qualitative and quantitative effects of this non-linearity, some of the

experiments with serotonin uptake were done at both 1 min and 4 min uptake times.

The presence of 100 μM pargyline in the uptake buffer should have protected the monoamine substrates from metabolism inside the synaptosomes. However, to confirm this assumption the ^{3}H-compounds associated with the synaptosomes were analyzed using ion exchange chromatography (10) after routine uptake assays with ^{3}H-serotonin and ^{3}H-dopamine. Less than 1.5% of the serotonin and dopamine present in the synaptosomes had been metabolized and metabolism was unaffected by the presence of VP.

Verapamil and D-600 were kindly supplied by Knoll Pharmaceutical Co., Whippany, New Jersey.

RESULTS

Inhibition of uptake by verapamil and D-600. Initial studies examined the effects of verapamil and D-600 on synaptosomal uptake measured at 40 nM substrate concentrations since only high affinity uptake is occurring to any significant extent at this concentration. As seen in Fig. 1, VP inhibited all of the uptake processes examined but the concentration required for inhibition varied markedly; the order of sensitivity to inhibition by VP was serotonin > dopamine > norepinephrine > choline. As seen in Fig. 2 the same order of effectiveness was seen with D-600. The IC_{50} values, obtained graphically from the data in Figs. 1 and 2, are shown in Table 1. Verapamil was more potent than D-600 at inhibiting serotonin and dopamine uptake but the two compounds were approximately equipotent at inhibiting norepinephrine and choline uptake.

Most of our studies employed whole forebrain as a source of synaptosomes since it provided a source of large quantities of material from a single animal and contained nerve endings for all of the uptake systems we wished to study. To more closely examine the specificity of the effects of VP, the corpus striatum was used as a source of predominantly dopaminergic synaptosomes and the cerebral cortex as a source of serotonergic and noradrenergic synaptosomes. Using these preparations the IC_{50} values for VP inhibition of serotonin, dopamine and norepinephrine uptake were determined and found to be virtually identical to those observed using forebrain synaptosomes (within ±10%, data not shown).

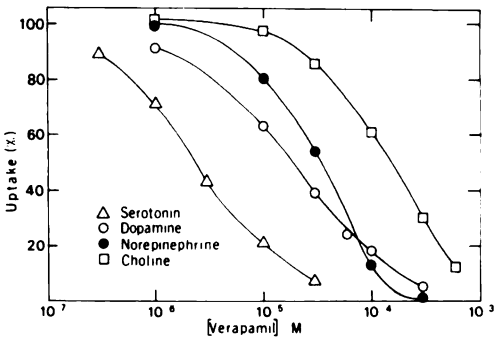

FIG. 1. *Inhibition of choline and neurotransmitter uptake into rat forebrain synaptosomes by different concentrations of verapamil*

Synaptosomes (50 μl in 0.32 M sucrose) from the equivalent of 6.5 mg of forebrain tissue were preincubated for 4 min at 37° in 0.4 ml of Krebs-Ringer phosphate buffer, pH 7.40, containing 100 μM pargyline, 1 mM ascorbic acid and the indicated concentrations of verapamil. Measurements of uptake were initiated by the addition of 50 μl of buffer containing ^{3}H-serotonin (△), ^{3}H-dopamine (○), ^{3}H norepinephrine (●), or ^{3}H-choline (□) such that the final concentrations of ligands in the uptake assays were 40 nM. Synaptosomes were allowed to accumulate the ligands for 4 min after which time the amount of ^{3}H accumulated by the synaptosomes was determined as described in METHODS. Each point represents the mean of 8 to 12 assays obtained with 2 or 3 different synaptosome preparations with the SEM less than 10% in all cases.

Characterization of the mechanism of inhibition by verapamil. Since serotonin uptake was the most sensitive to VP (and thus potentially the most important to the physiological effects of the drug) the various parameters for the effect of VP on this uptake system were studied in detail. Considering the structural differences between the four substrates being studied it seemed unlikely that VP and D-600 could be acting as competitive inhibitors of the serotonin uptake system. Direct evaluation of the kinetic mechanism of inhibition is seen in Fig. 3. At low concentrations of VP (below IC_{50}), kinetic analysis was most consistent with a non-competitive inhibitory effect. However, at higher concentrations of VP

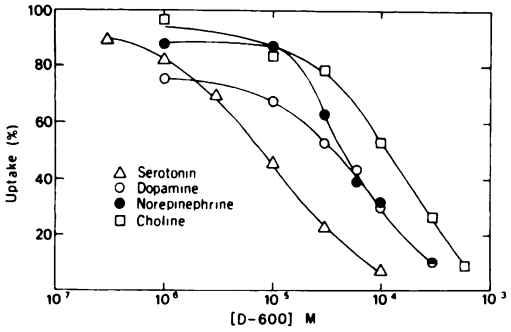

FIG. 2. *Inhibition of choline and neurotransmitter uptake into rat forebrain synaptosomes by different concentrations of D-600*

Uptake of ^{3}H-substrates (40 nM) into synaptosomes was measured as described in the legend to Fig. 1 using ^{3}H-serotonin (△), ^{3}H dopamine (○), ^{3}H-norepinephrine (●), or ^{3}H-choline (□). Each point represents the mean of 4 to 8 assays with SEM less than 10%.

TABLE 1

Concentrations (IC_{50}) of verapamil or D-600 required to inhibit high affinity synaptosomal uptake by 50%

The data in Figs. 1 and 2 were used to estimate the concentration of verapamil and D-600 required to inhibit uptake by 50%.

Substance being transported	IC_{50}	
	Verapamil	D-600
	(μM)	
Serotonin	2.3	8.5
Dopamine	18	37
Norepinephrine	32	44
Choline	150	120

an unusual relationship was observed that was not consistent with either non-competitive or competitive mechanisms. Similar kinetic results at low and high concentrations of VP were obtained for norepinephrine and choline uptake, i.e., an apparently noncompetitive inhibitory effect at low levels of inhibition but less definable kinetics at high levels of inhibition (data not shown).

As previously noted in METHODS, serotonin uptake was not linear during the 4 min assay routinely used. In order to determine if this non linearity of the assay system was affecting our results, the IC_{50} value and competitive versus non-competitive nature of the inhibition was evaluated using a 1 min uptake time. The apparent IC_{50}

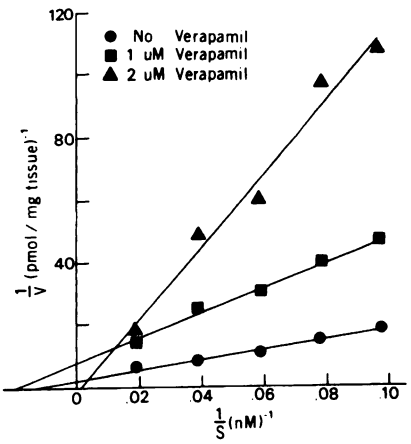

FIG. 3. *Double reciprocal analysis of inhibition of serotonin uptake into rat forebrain synaptosomes by verapamil*

Uptake of serotonin into synaptosomes was measured as described in METHODS at serotonin concentrations between 10 and 50 nM. The concentrations of verapamil were: ●— no verapamil, ■—1 μM verapamil, ▲—2 μM verapamil. Each point represents the mean of 4 assays with SEM less than 10%.

based on the 1 min assay was only slightly lower (1.7 μM) than that observed at 4 min (2.3 μM) and inhibition still appeared to be non-competitive at low concentrations and difficult to define at high concentrations of VP (data not shown). In addition, the extent of inhibition by VP was evaluated as a function of time (Table 2) and found to be virtually constant over a 10 min period. Thus, the apparent non-linearity of serotonin uptake with time had little effect on our observed inhibition of uptake by VP.

The data in Table 2 suggested that the effect of VP was exerted very rapidly. However, routine assays such as those depicted in the table employed a 4 min preincubation (either with or without verapamil) prior to the uptake time. To examine more closely the time course of the initiation of inhibition the uptake of serotonin in a short 1 min uptake time was examined following various preincubation times (Table 3). The effect of verapamil was seen even in the absence of preincubation but was not maximized on a percentage basis until at least 1 min of preincubation. Thus, the effect of VP was rapid but not instantaneous.

Another important kinetic question is whether the effect of VP is reversible and,

TABLE 2

Time dependence of verapamil inhibition of serotonin uptake

The uptake of 40 nM serotonin was measured as described in METHODS in either the absence or presence of 1.5 μM VP. Each value represents the mean ± SEM for 4 assays.

Time	Serotonin uptake		Inhibition by verapamil
	0 verapamil	1.5 μM verapamil	
(min)	*(fmole/mg tissue)*		*(%)*
1	170 ± 3	105 ± 3	38
2	248 ± 6	135 ± 3	46
3	310 ± 6	168 ± 8	46
4	361 ± 7	205 ± 5	43
6	423 ± 7	262 ± 9	38
10	521 ± 7	358 ± 8	31

TABLE 3

Time required for onset of action of verapamil

Uptake of 40 nM ^{3}H-serotonin was measured as described in METHODS except that the initial preincubation time in the presence of verapamil was varied from 0–10 min, as indicated, and time of exposure to ^{3}H-serotonin was decreased to 1 min. Each value represents the mean ± SEM of 4 assays.

Preincubation time	Serotonin uptake		Inhibition by verapamil
	No verapamil	2 μM verapamil	
(min)	*(fmole/mg tissue)*		*(%)*
0	57.1 ± 3.2	23.4 ± 1.2	59
1	88.5 ± 2.4	25.3 ± 0.4	72
4	97.0 ± 4.1	25.4 ± 1.1	74
10	91.7 ± 4.1	21.3 ± 1.0	77

if so, how rapidly. Two different experiments were designed to test reversibility and both demonstrated that reversal is as rapid as initiation of inhibition. In the first experiment, synaptosomes were pretreated at 37° in 0.32 M sucrose either with or without 2 μM VP and then the concentration of VP was diluted to 0.2 μM by addition of the synaptosomes to the uptake assays. As seen in Table 4, the rate of serotonin uptake returned to an uninhibited value virtually instantaneously. Preincubation in the absence of VP was not required to reverse the inhibition caused by VP.

In a second experiment testing reversibility synaptosomes were pretreated in KRP for 10 min at 37° either with or without 2 μM VP. The synaptosomes were then

TABLE 4

Time required for reversal of VP inhibition of serotonin uptake following pretreatment in 0.32 M sucrose

Synaptosomal uptake of 40 nM ^{3}H-serotonin was measured as described in METHODS except that synaptosomes in 0.32 M sucrose were first pretreated with either no VP or 2 μM VP at 37° for 4 min, returned to the ice bath, and then uptake in 1 min was determined after preincubation for 0–10 min as indicated. Since synaptosomes pretreated with 2 μM VP would contribute 0.2 μM VP to uptake assays, 0.2 μM VP was also added to assays with synaptosomes pretreated without VP. Each value represents the mean ± SEM for 4 assays. Uptake in the absence of VP averaged 83.1 fmole/mg tissue and in the presence of 2 μM VP 23.8 fmole/mg tissue.

Preincubation time	Serotonin uptake	
	Synaptosome pretreatment	
	No verapamil	2 μM verapamil
(min)	*(fmole/mg tissue)*	
0	55.2 ± 3.2	52.0 ± 1.1
1	69.1 ± 1.4	65.9 ± 0.2
4	68.5 ± 2.5	62.4 ± 1.4
10	65.0 ± 0.7	57.8 ± 5.1

cooled, diluted with 3 volumes of cold 0.32 M sucrose with or without 2 μM VP and isolated using centrifugation. The pelleted synaptosomes were then resuspended in cold sucrose without VP and serotonin uptake in 4 min evaluated following various preincubation times in the absence or presence of VP (Table 5). Unlike the results shown in Table 4, the inhibitory effects of VP were still partially apparent following 1 min of preincubation time. However, with 5 min of preincubation the effect of VP was completely reversed. Thus, pretreatment of synaptosomes with VP in buffer required somewhat longer to reverse but reversal still was accomplished within a matter of minutes, demonstrating that the effects of VP are rapidly reversible.

The primary mode of action of VP has been thought to reside in its ability to block Ca^{++} movements. To test the hypothesis that this was its effect on synaptosomes, increasing concentrations of $CaCl_2$ were added to the uptake assays and the effects on serotonin uptake studied (Table 6). Increasing $CaCl_2$ in the absence of VP substantially inhibited serotonin uptake. However, the inhibitory effect of VP was vir-

TABLE 5

Time required for reversal of verapamil inhibition of serotonin uptake following pretreatment in buffer

Synaptosomes were pretreated for 10 min in KRP at 37° either in the presence or absence of 2 μM VP. Three volumes of cold 0.32 M sucrose then were added (VP concentration being maintained as in the pretreatment) and the synaptosomes isolated by centrifugation. Synaptosomes were then resuspended in 0.32 M sucrose without VP and used to measure serotonin uptake following indicated preincubation times. Each point represents the mean ± SEM of 4 assays.

Verapamil concentration		Preincubation time	Seotonin uptake
Pretreatment	Uptake		
(μM)		(min)	(fmole/mg protein)
0	0	1	275 ± 15
2.0	0[a]	1	148 ± 19
0	2.0	1	90 ± 10
2.0	2.0	1	102 ± 22
0	0	5	328 ± 5
2.0	0	5	314 ± 5
0	2.0	5	131 ± 10
2.0	2.0	5	129 ± 12
0	0	10	388 ± 12
2.0	0	10	323 ± 13
0	2.0	10	165 ± 4
2.0	2.0	10	165 ± 5

[a] The concentration of VP contributed by synaptosomes pretreated with VP was estimated to be approximately 2 nM, which is insufficient to cause any inhibition of serotonin uptake.

TABLE 6

The effect of Ca^{++} concentration on inhibition of serotonin uptake by verapamil

Uptake of 40 nM ^{3}H-serotonin into synaptosomes was measured as described in METHODS except that the indicated concentrations of $CaCl_2$ were present during the 4 min preincubation and 4 min uptake. Each value represents the mean ± SEM of 4 assays.

[$CaCl_2$]	Serotonin uptake		Inhibition by verapamil
	0 verapamil	2 μM verapamil	
(mM)	(fmole/mg tissue)		(%)
1.27	266 ± 6	93.1 ± 0.8	65
2.54	222 ± 6	89.3 ± 1.5	60
6.35	182 ± 8	78.0 ± 1.1	57
12.7	161 ± 6	60.5 ± 2.9	62

tually unchanged on a percentage basis by elevation of $CaCl_2$. In other experiments, removal of $CaCl_2$ from the uptake medium along with the addition of 0.2 mM EGTA had no effect on either the uninhibited rate of serotonin uptake or the extent of inhibition by VP (data not shown).

In addition to inhibition of Ca^{++} movements, VP has been shown to block Na^+ movements through "slow" channels (6, 7). Furthermore, all of the uptake systems being studied have been shown previously to be dependent on extracellular Na^+. For these reasons we examined the effect of varying the NaCl concentration (substituting sucrose to maintain osmolarity) on the inhibition of serotonin uptake by VP (Fig. 4). Decreasing NaCl to 40 mM and 20 mM inhibited uptake from 171 fmole/mg tissue to 153 and 128 fmole/mg tissue, respectively. More importantly, at 40 mM NaCl the serotonin uptake system was approximately twice as sensitive to inhibition by VP as at 135 mM NaCl (IC_{50} = 1.4 μM and 3.0 μM, respectively). Further decreasing NaCl to 20 mM caused a slightly greater inhibition by 0.3 μM VP but no change in the amount of inhibition at higher VP con-

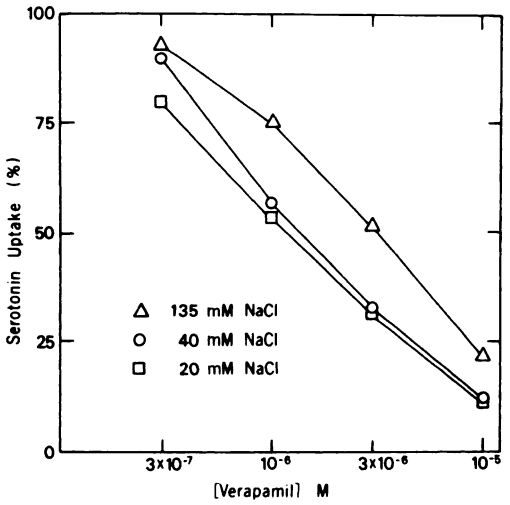

FIG. 4. *Inhibition of serotonin uptake into synaptosomes by verapamil at varying concentrations of NaCl.*

Uptake of serotonin (40 nM) into synaptosomes was measured as described in METHODS in buffer containing 135 mM, 40 mM or 20 mM NaCl. Each point represents the mean of 8 to 12 assays using 2 or 3 different synaptosome preparations. Uninhibited rates of uptake were 171, 153 and 128 fmole/mg tissue at 135, 40 and 20 mM NaCl, respectively.

centrations. Thus, decreasing the NaCl concentration increased inhibition of serotonin uptake by VP but only to a limited extent.

DISCUSSION

Our results do not allow an easy explanation as to the mechanism of action of VP in our system. Verapamil does not appear to be causing any major disruptive effect on the synaptosomes because the effects of the drug are rapidly and completely reversible. The possibility that VP and D-600 compete directly with the different substrates for their uptake systems seems unlikely since the kinetic evaluation of inhibition bears no resemblance to a competitive interaction (Fig. 3).

A more likely explanation of our results would be that VP is interfering with the movement of Ca^{++} and/or Na^+ since the drug has been shown to inhibit the movements of these ions through certain types of ion channels (1, 3, 4, 6, 7). The possibility that VP is working through an antagonism of Ca^{++} influx seems less likely for several reasons. To begin with, the neurotransmitter uptake systems have been observed to be independent of external Ca^{++}, although White was able to show a small inhibition of norepinephrine uptake upon removal of divalent cations (11). Under our assay conditions removal of Ca^{++} with the addition of EGTA had no effect on serotonin uptake. In addition, elevation of Ca^{++} actually inhibited uptake (Table 6). The inability of increased Ca^{++} to reverse inhibition by VP (Table 6) is further evidence against an interaction between VP and Ca^{++} influx; however a non-competitive interaction between VP and Ca^{++} could not be ruled out by this experiment.

In contrast to Ca^{++}, Na^+ is very important to high affinity uptake systems. Since VP is known to interfere with Na^+ movement in "slow" channels (6, 7), the possibility arises that VP also is interfering with the Na^+-dependent component of the neurotransmitter uptake systems. This hypothesis would imply that "slow" Na^+ channels and the Na^+-dependent component of the neurotransmitter uptake systems are in some way similar. Unfortunately, very little is known about the "slow" Na^+ channel which makes a critical evaluation of this hypothesis difficult. The Na^+-dependency of the neurotransmitter uptake systems has been more extensively studied but again little is known as to the actual mechanism by which Na^+ is required. Some authors feel that the Na^+ gradient across the neuronal membrane acts as the driving force for concentrative uptake (12, 13), but other investigators do not believe that such a role adequately accounts for the effects of Na^+ (14). Choline uptake has been studied extensively and the proposal made that choline and Na^+ are co-transported (15). None of these possibilities would preclude a structural or functional similarity between "slow" channels and a component of the uptake systems.

The possibility that VP was interfering with or blocking some Na^+ transport site involved in the uptake systems was tested by varying the NaCl concentration in the uptake medium. As would be expected if Na^+ and VP were competing for the same site, decreasing NaCl increased the ability of VP to block serotonin uptake. However, the change, although very reproducible, was small and suggested that if VP is affecting Na^+ movement required for uptake it is doing so in a manner in which it does not directly compete with Na^+ for a given site. This non-competitive interaction between Na^+ and VP is consistent with a previously demonstrated non-competitive interaction between Ca^{++} and VP (16).

The differences in the IC_{50} values for inhibition of the different uptake systems are difficult to explain. The IC_{50} values do seem to reflect the sensitivities of the actual uptake systems rather than the sensitivity of a general uptake system transporting different substrates. Evidence for this comes from our observation that the IC_{50} for a given substrate is the same whether determined in a whole forebrain preparation or a preparation from a region enriched for specific nerve terminals. Therefore, the different sensitivities of the uptake systems to VP must reflect either a different mode of inhibition with each system or the variable characteristics of some common element being affected by the drug.

A final possible explanation for our re-

sults is that VP actually is working by stimulating release rather than inhibiting uptake. This possibility seems very unlikely since Murakami *et al.* have shown that concentrations of VP even as high as 100 μM cause only a very small amount of serotonin to be released from rat brain slices (17). Also, Cohen and Gutman have shown that 1 mM VP, which completely blocks acetylcholine-induced release of catecholamines from adrenal medulla, has no effect on the unstimulated release of catecholamines from this tissue (18).

Regardless of the mechanism by which VP and D-600 inhibit uptake processes, this effect of these drugs must be examined for physiological significance since they are in use clinically in some countries. Mannhold *et al.* recently have performed an extensive study of the concentration dependence of the inhibition of papillary muscle contraction by VP and several of its analogues *in vitro* (19). In their studies the ED_{50} for VP was found to be 3.5 μM, a value very close to that observed for the inhibition of serotonin uptake in our studies. On the other hand, D-600 was more potent than VP at inhibiting papillary contraction (ED_{50} = 1.2 μM) whereas in our system D-600 was less potent than VP. The similar IC_{50} values in our study and that of Mannhold *et al.* suggest that the phenomenon being observed in the two systems is quite similar in some way, but the reversed order of potency between VP and D-600 in our system suggests that a significantly different structural requirement exists between the two systems. Although this difference would be consistent with a slight difference between a primarily Na^+-conducting site and a Ca^{++}-conducting site as we propose, a detailed structure activity relationship must be examined before more can be said about this possibility.

A second important point regarding physiological importance is that we are studying the effects of VP in central nervous system tissue and VP's effects have been thought to reside in peripheral tissues. It is very likely that VP also would inhibit neurotransmitter uptake systems at peripheral nerve endings since the basic uptake process seems to be essentially identical throughout the nervous system. In addition, the lipophylic nature of both VP and D-600 would lead one to predict that the drugs would easily pass the blood brain barrier and distribute into the brain.

It is clearly premature to draw any conclusions on the relationship between our observations and the physiological actions of VP. However, the few studies that we have been able to locate (20, 21) indicate that the serum concentration of VP achieved clinically probably is somewhere in the range of 1 μM. Since our studies indicate that at least serotonin uptake systems will be affected by this concentration of the drug, the question of whether or not such an inhibition is occuring *in vivo* becomes an important one which warrants additional investigation.

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