INHIBITION OF SYNAPTOSOMAL CHOLINE UPTAKE BY NAPHTHYLVINYLPYRIDINIUMS

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1. Introduction

A carrier system for the transport of choline into synaptosomes has been demonstrated by several investigators [1–3]. Recently, evidence has been presented for the existence of high and low affinity components in this transport system [4–8]. The high affinity component is Na^+-dependent [4, 7], is competitively inhibited by hemicholinium-3 [5, 6], and appears to be that which supports acetylcholine (ACh) synthesis [4, 7, 8]. The latter observation suggests that the transport of choline by the high affinity system may be linked to the activity of choline acetyltransferase (ChAc, EC 2.3.1.6). To test this hypothesis we have investigated the effect of naphthylvinylpyridinium inhibitors of ChAc on the synaptosomal uptake of choline. Aside from being inhibitors of ChAc, these agents inhibit drug metabolizing enzymes [9], potentiate the excitatory effects of amphetamines [9], and impair passive avoidance behavior [10]. Although these agents are potent *in vitro* inhibitors of ChAc, they do not decrease whole brain or hippocampal levels of ACh [10–12].

Naphthylvinylpyridinium agents inhibit synaptosomal choline uptake. The mechanism of inhibition appears to be independent of an action on ChAc.

2. Materials and methods

All chemicals used were reagent grade. *N*-methyl-[^{3}H]choline, S.A. 16Ci/mM, was purchased from Amersham/Searle and purified by chromatography on Sephadex SE-25 (Pharmacia). Naphthylvinylpyridine HCl, methiodide and *N*-hydroxyethyl bromide were synthesized by the method of Cavalitto et al. [13, 14]. Hemicholinium-3 was generously provided by Dr. V.B. Haarstad, Department of Pharmacology, Tulane University, New Orleans, La, USA.

2.1. *Isolation of synaptosomes*

A crude synaptosomal fraction was isolated from rat forebrain as described by Whittaker and Barker [15].

2.2. *Synaptosomal uptake of [^{3}H]choline*

The crude synaptosomal fraction was used without further purification since several investigators have shown that choline uptake by this fraction is primarily due to synaptosomes [1, 4, 7, 8]. Synaptosomes from 20 mg tissue (1200 μg protein) were suspended in 1 ml of Krebs–Ringer phosphate buffer (pH 7.5) [16], containing 5 nmol of [^{3}H]choline (S.A. 0.17 or 1 μCi/nmol). The choline concentration (5×10^{-6} M) selected was one in which choline uptake by the high and low affinity components would be approximately equal [7, 8]. Incubations were initiated by transferring the tubes to a 37°C water bath and terminated after 5 min by transfer to an ice bath. Synaptosomes from 4 or 8 mg tissue were collected on a 25 mm Millipore cellulose acetate filter (0.8 μ) and washed with 10 ml of ice cold Krebs–Ringer phosphate buffer. The filters were transferred to scintillation vials and solubilized with 1 ml of cellosolve. Ten ml of Triton X-100-toluene cocktaile [17] were added and the samples were counted in a Beckman liquid scintillation spectrometer

Table 1
Inhibition of synaptosomal [^{3}H]choline uptake by hemicholinium-3 and napththylvinylpyridiniums*.

Drug**	Concn. (μM)	Uptake***	Percent inhibition	K_i**** ChAc
Control	–	889.5	0	–
HC-3	50	139.1	82	–
NVP	50	323.0	64	2.2×10^{-5}
NVPM	50	300.0	66	3.3×10^{-6}
NVPH	50	441.5	51	1×10^{-6}

* Activity determined as described in text.
** Abbreviations: hemicholinium-3, HC-3; naphthylvinylpyridine HCl, NVP; naphthylvinylpyridinium methiodide, NVPM; hydroxyethyl-naphthylvinylpyridinium bromide, NVPH.
*** dpm/mg Tissue/5 min. The specific activity of [^{3}H]choline was 0.17 μCi/nmol.
**** From Cavallito et al. [13, 14].

In some experiments synaptosomes from 100 mg of tissue were incubated in 5 ml of buffer as described above and isolated by centrifugation at 340 000 *g*/min. The [^{3}H]ACh and [^{3}H]choline in the pellet were extracted, separated, and determined as described by Barker et al. [17]. Non-specific and diffusional uptake were corrected by subtracting values obtained at 0°C from those at 37°C.

2.3. *Analytical procedures*

Protein was determined by the method of Lowry et al. [18], using bovine serum albumin as a standard.

3. Results

The choline acetyltransferase inhibitors, naphthylvinylpyridine (VNP), naphthylvinylpyridinium methiodide (NVPM) and hydroxyethyl-naphthyl-vinylpyridine bromide (NVPH) inhibit the uptake of [^{3}H]choline by synaptosomes. Initial experiments (table 1) showed the order of inhibitory effect of NVPM $\geqslant$ NVP $>$ NVPH; but all were less potent than hemicholinium-3 (HC-3). Subsequent experiments dealt only with NVPH and HC-3. In fig. 1, the ability of NVPH to block [^{3}H]choline uptake is compared to that of HC-3 over a wide dose range. As in the initial experiments, it is seen HC-3 is much more potent than NVPH. Also the shape of the log dose–response curves are very different. With HC-3 at 1–10 μM the inhibition is relatively constant, around 60%–70%. The K_i for HC-3 on the high affinity component has been reported to be 0.05–0.1 μM [4]. Thus at the lowest concentration of HC-3 (1 μM), used here, the high affinity transport component should be completely blocked. The I_{50} to inhibit the remaining 40%

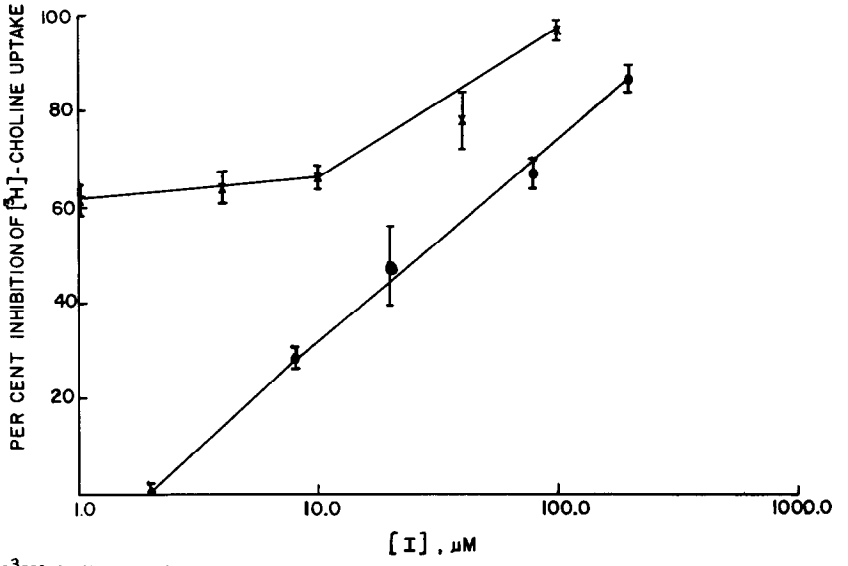

Fig. 1. Inhibition of [^{3}H]choline uptake by NVPH (•) and HC-3 (x). Values are mean ± SEM (N = 3). Control uptake was 10 100 ± 74 dpm/mg tissue/5 min. The specific activity of [^{3}H]choline was 1 μCi/nmol.

Table 2
Inhibition of synaptosomal [^{3}H]choline uptake and [^{3}H]acetylcholine synthesis*.

Drug	Concn. (μM)	[^{3}H]Choline Uptake**	[^{3}H]Acetylcholine synthesis**
Experiment 1***			
Control	–	342.3 ± 23.8	104.3 ± 7.7
HC-3	10	44.4 ± 9.6	0.1 ± 0.3
NVPH	20	148.6 ± 48.1	90.5 ± 2.0
Experiment 2			
Control	–	353.2 ± 21.0	89.3 ± 1.7
NVPH	20	223.2 ± 19.0	67.9 ± 2.2

* Activity determined as described in text. Data are mean ± SEM (N = 3).
** dpm/mg Tissue/5 min. Specific activity of choline was 0.17 μCi/nmol.
*** Physostigmine (5×10^{-4}M) present in extractant for experiment 1, but not for experiment 2.

of the uptake, presumably that due to the low affinity component, is about 45 μM, in good agreement with a value of 50 μM, reported by Haga and Noda [4] for the Na^+-independent (low affinity) component of choline transport. In contrast, the inhibition produced by NVPH from 2–200 μM increases in an apparent linear manner with the log of the dose at the concentration of choline used in these experiments.

In table 2 the effect of NVPH and HC-3 on transport of [^{3}H]choline and formation of [^{3}H]acetylcholine is shown. Here it is seen that NVPH at 20 μM is more effective in blocking [^{3}H]choline uptake than it is in blocking the formation of [^{3}H]ACh. With HC-3 at 10 μM, there was no detectable formation of [^{3}H]ACh, as would be expected for a complete inhibition of the high affinity uptake system [4, 7, 8].

4. Discussion

The naphthylvinylpyridinium inhibitors of ChAc block synaptosomal uptake of choline by a means which appears to be unrelated to their ability to inhibit ChAc *in vitro*. Firstly the potency in blocking uptake does not parallel their ability to inhibit ChAc (table 1). Secondly, the inhibition curves (fig. 1) show that NVPH blocks both the high and low affinity components of the choline transport system. The greater inhibition of choline transport compared to choline incorporation into ACh (table 2) indicates some selectivity in blocking the low affinity component. By contrast, with HC-3 the reverse obtains, i.e., blockade of choline uptake is equal to or less than the block of ACh synthesis [4] because HC-3 is selective for the high affinity uptake component [4, 6].

Further, since the formation of ACh by synaptosomes is only slightly inhibited by NVPH at 20 μM (20 × the K_i for ChAc) [13], it would appear that the synaptosomal membrane is relatively impermeable to NVPH. This may be the reason why NVPM [10] and its analogs [11, 12] fail to decrease brain levels of ACh.

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