

The neurotoxin histrionicotoxin interacts with the putative ion channel of the nicotinic acetylcholine receptors in the central nervous system

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Perhydrohistrionicotoxin at micromolar concentrations blocked the nicotine-evoked transmitter release from perfused striatal (dopaminergic) and hippocampal (cholinergic) nerve terminals. Perhydrohistrionicotoxin failed to compete with [³H]nicotine for its high-affinity binding site in rat brain, suggesting that the action of this toxin on central nicotinic receptors is noncompetitive. From the dose-response curve, 50% inhibition of nicotine-evoked striatal dopamine release occurred at 5 μ M perhydrohistrionicotoxin, a value similar to that obtained in frog sartorius muscle and *Electrophorus* electroplax. This close agreement may suggest that the ionic channel of the presynaptic nicotinic acetylcholine receptor of brain neurons has similar properties to those of the peripheral receptor.

Neuronal nicotinic acetylcholine receptor; Ion channel; Histrionicotoxin; Perhydrohistrionicotoxin

1. INTRODUCTION

Histrionicotoxin (HTX) is a spiropiperidine alkaloid which was originally isolated from the skin secretion of the Colombian frog, *Dendrobates histrionicus* [1]. It specifically blocks the ion channel of the nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction [2], on electroplax [3], cultured muscle [4], and adrenal medullary [5] cells. Additionally, the toxin reduces the spontaneous discharge induced by local application of acetylcholine in cerebral cortex and spinal cord in the cat [6]. Nicotinic receptors are known to be present in the central nervous system [7], and while it appears that they are genetically related to those in the periphery [8,9] and similar effects of HTX might be expected, there have been no direct

studies of this question. Early reports on ganglionic nAChRs suggested parallel conductance behavior with the well characterized peripheral receptor [7], but more recent studies on the putative nAChR in chick optic lobe suggest that binding of the HTX analog perhydrohistrionicotoxin (H₁₂HTX) is not totally specific [10].

We have been investigating two particular classes of central presynaptic nAChRs that facilitate neurotransmitter release, one a heteroreceptor on striatal dopaminergic terminals [11,12] and the other, an autoreceptor on hippocampal terminals [13]. The striatal receptor has different properties, in terms of its sensitivity to neosurugatoxin and α -bungarotoxin, from the peripheral nAChR [12]. Recent observations (Wonnacott, unpublished) demonstrate that the hippocampal autoreceptor is also largely insensitive to α -bungarotoxin when stimulated with low (1–10 μ M) concentrations of nicotine, but highly sensitive to the agonist toxin (+)anatoxin [14]

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(Wonnacott and Albuquerque, unpublished). The aim of the present study was to establish whether these two central nAChRs have similar ion channel characteristics to the peripheral nAChR in spite of differences in their recognition sites. In the experiments described here, we have examined the effects of H₁₂HTX on the nicotine-induced release of dopamine and acetylcholine from striatal and hippocampal nerve terminals, respectively. A preliminary account of some aspects of this work has been presented [15].

2. MATERIALS AND METHODS

2.1. Synaptosome perfusion

Striatal and hippocampal synaptosomes were prepared from adult male Wistar rat brain as described [15]. Striatal synaptosomes were incubated at 37°C in Krebs bicarbonate medium containing [³H]dopamine (spec. act. 46 Ci/mmol; 0.1 μM final concentration) for 5 min. Hippocampal synaptosomes were incubated with [³H]choline (spec. act. 39 Ci/mmol; final concentration 0.8 μM) for 20 min. Synaptosomes were then washed by centrifugation in a bench centrifuge (800 × g, 7 min) and perfusion was carried out exactly as described [12]. Stimulation of synaptosomes was achieved by the introduction of a pulse (100 μl) of (–)nicotine (1 or 10 μM) into the perfusion line; stimulation was carried out at 40, 80 and 120 min after the start of the perfusion. The responses are designated S₁, S₂ and S₃, respectively. The effects of H₁₂HTX on transmitter release were tested by introducing the toxin at the appropriate concentration into the perfusion line 65 min after perfusion started. In some experiments H₁₂HTX was present throughout the remainder of the perfusion, in others it was present only for 30 min. Results for transmitter release are presented by calculating the second and third responses to nicotine as a percentage of the first response.

The binding of (–)-[³H]nicotine to washed P₂ membranes from whole rat brain was measured as described [12,17].

2.2. Drugs and toxins

[7,8-³H]Dopamine, [methyl-³H]choline chloride and (–)-(N-methyl-³H)nicotine were purchased

from Amersham (Bucks, England). (–)Nicotine was from Sigma (Poole, England). Perhydrohistrionicotoxin was stored in ethanol at 10 mM at –20°C and samples were diluted in bicarbonate buffer immediately prior to perfusion. Ketamine was a gift from Parke-Davis Co. (Cambridge, England). A stock solution (10 mM in water) was stored at 4°C [17] and further diluted in bicarbonate buffer immediately prior to use.

3. RESULTS

The release of [³H]dopamine and [³H]acetylcholine from striatal and hippocampal synaptosomes respectively was monitored by perfusion. Typical release profiles are shown in fig. 1. After an initial washout period of 40 min the basal release stabilized. Stimulation with K⁺ (16 mM) or nicotine (1 or 10 μM) provoked a peak of release representing approx. 1% of the radioactivity taken up by the tissue. A second stimulation (S₂) after an interval of 30 min resulted in a response that was consistently 85–90% of the initial (S₁) response (table 1). In the presence of H₁₂HTX (5 μM), the S₂ response to nicotine was diminished. Expressing the S₂/S₁ ratio for perfusions in the presence of H₁₂HTX as a percentage of the ratio for parallel experiments in which the toxin was absent, it was seen that dopamine release in response to 10 and 1 μM nicotine was reduced by 33 and 64%, respectively. Under identical experimental conditions the K⁺-evoked dopamine release was not significantly altered (table 1). Because of the lower uptake of [³H]choline by hippocampal synaptosomes and its incomplete acetylation, lower levels of tritium are released from the hippocampal preparations (fig. 1). This necessitates the use of higher nicotine concentrations in order to ensure accurately quantifiable peaks. Acetylcholine release in response to 10 μM nicotine was inhibited by 28% by 4 μM H₁₂HTX, a figure that is very close to the value obtained in the striatal preparations with similar concentrations.

When striatal synaptosomes were subjected to a third stimulation by 1 μM nicotine in the continued presence of H₁₂HTX at 5 μM no further inhibition of the response to nicotine was produced. The S₃/S₁ ratio was 35.3 ± 4.7 (n = 3) which agrees well with the S₂/S₁ ratio (table 1). However if exposure to H₁₂HTX was terminated after the second

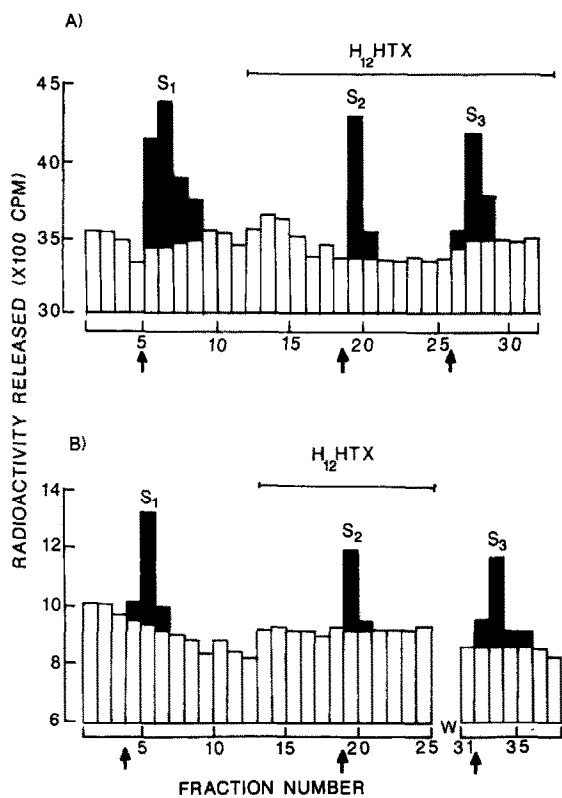


Fig.1. Perfusion profiles showing the release of radioactivity from (A) striatal and (B) hippocampal synaptosomes loaded with [3 H]dopamine and [3 H]acetylcholine, respectively. Synaptosomes incubated with radiolabelled transmitter or precursor were perfused at a flow rate of 9 ml/h for a 40 min washout period prior to the collection of 8 drop (300 μ l) fractions. The synaptosomes were stimulated with successive pulses (100 μ l) of (–)nicotine at 40, 80 and 120 min, indicated by the arrows. The responses are designated S₁, S₂, S₃; shaded areas represent the evoked release above basal release. (A) Striatal synaptosomes loaded with [3 H]dopamine were stimulated with 1 μ M nicotine; the concentration of H₁₂HTX was 5 μ M. (B) Hippocampal synaptosomes loaded with [3 H]choline were stimulated with 10 μ M nicotine; the concentration of H₁₂HTX was 4 μ M.

stimulation and the synaptosomes subsequently perfused with normal medium, the S₃/S₁ ratio approached control levels: values for stimulation with 10 μ M nicotine were 98.0 ± 2.5 and $89.8 \pm 1.6\%$ of control preparations for striatal and hippocampal synaptosomes, respectively.

The effect of increasing concentrations of H₁₂HTX on 1 μ M nicotine-evoked striatal

Table 1

The effect of H₁₂HTX and ketamine on nicotine- and K⁺-evoked transmitter release

Synaptosome preparation	Stimulus	S ₂ /S ₁ (%)		
		Control	H ₁₂ HTX	Ketamine
Striatum	nicotine (1 μ M)	86.3 ± 5.1 (5)	41.3 ± 4.6 (7)	55.3 ± 5.8 (4)
	nicotine (10 μ M)	84.0 ± 6.8 (4)	56.0 ± 2.1 (2)	–
	K ⁺ (16 mM)	86.0 ± 5.1 (2)	76.0 ± 3.5 (2)	91.5 ± 0.5 (2)
Hippocampus	nicotine (10 μ M)	89.1 ± 4.4 (4)	67.3 ± 1.8 (4)	–

Striatal and hippocampal synaptosomes preloaded with [3 H]dopamine and [3 H]choline, respectively, were stimulated with pulses (100 μ l) of (–)nicotine or K⁺. The release of radiolabel evoked by a second pulse (S₂) is expressed as a percentage of release in response to the initial stimulation (S₁). H₁₂HTX (5 μ M, striatal preparations; 4 μ M, hippocampal preparations) or ketamine (1 μ M) were introduced 15 min prior to S₂. Results are means \pm SE or range of the number of determination given in parentheses

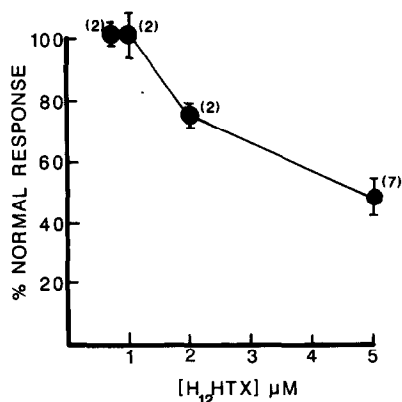


Fig.2. Inhibition of nicotine (10 μ M)-evoked release of [3 H]dopamine for striatal synaptosomes by H₁₂HTX. Striatal synaptosomes were repeatedly stimulated with nicotine (10 μ M), initially in the absence and then either in the absence or presence of H₁₂HTX (fig.1). The S₂/S₁ ratio obtained in the presence of the toxin was expressed as a percentage of the control S₂/S₁ value. Vertical bars indicate the range or SE of the number of determinations given in parentheses.

[3 H]dopamine release was examined (fig.2). A steep dose response curve over a narrow range of toxin concentrations was observed; an IC₅₀ value of 5.0 μ M was derived. To determine if the observed antagonism by H₁₂HTX was competitive or noncompetitive, the toxin was examined in competition binding experiments. Over the concentration range effective in blocking nicotine-evoked [3 H]dopamine release (fig.2) H₁₂HTX had no effect on (–)-[3 H]nicotine binding to rat brain membranes.

The anesthetic ketamine was also treated on the striatal preparations. At micromolar concentrations ketamine markedly reduced the response to 1 μ M nicotine but had no effect on the K⁺-evoked release (table 1).

4. DISCUSSION

Evidence is accumulating from molecular genetics for the presence on neurones of nAChR having an overall structure similar to that of the well characterized peripheral nAChR [7,8]. Direct studies of the nAChR in brain have concentrated on the pharmacology of the ligand binding site and such studies point to marked differences in the specificity of this site compared with the peripheral

nAChR [11]. However, there has been little attention given to the nature of the presumed ion conductance channel of the central nAChR and its relationship to its peripheral counterpart [10,15]. H₁₂HTX at micromolar concentrations was found to block nicotine-evoked transmitter release from striatal (dopaminergic) and hippocampal (cholinergic) nerve terminals (fig.1; table 1). HTX and its perhydro derivative are noncompetitive inhibitors of the peripheral nAChR, and electrophysiological measurements demonstrate that the toxins achieve this effect by specific blockade of the ion channel of nAChR [19]. Such an effect evolved primarily from the interactions of these toxins with the nAChR inducing desensitization and blockade of the receptor in closed conformation. These actions of H₁₂HTX are similar to those of the phenothiazines [20] and meproadifen [21]. The noncompetitive action of H₁₂HTX or the nAChR of the CNS is confirmed by the failure of the toxin to compete with [3 H]nicotine for its high-affinity binding site. In frog sartorius muscle [18], the concentration of H₁₂HTX producing 50% blockade was reported to be 5 μ M and 0.8 μ M in *Electrophorus* electroplax [3]. The dose-response curve determined in the present study (fig.2) yields an IC₅₀ value of 5.0 μ M for the inhibition of nicotine-evoked striatal dopamine release. The close agreement between this value and those obtained for peripheral nAChR strongly suggests that the presynaptic nAChR on brain neurones acts through a similar ionophore. The time course for the action of H₁₂HTX on the dopamine release system is similar to that reported in electroplax [3], the maximum blockade of the nicotine effect is seen after 15 min (fig.1A) as compared with a time of 15–25 min in electroplax. In agreement with this study [3], we also observe an almost complete recovery of the normal response following a 15 min washout (fig.1B). The anesthetic ketamine also interacts with the ion channel of the peripheral nAChR [17]. The inhibition of nicotine-evoked dopamine release by a relatively low concentration of ketamine in the present study (table 1) is further evidence for the presence of ion channel in neuronal nAChR. This is compatible with the recent evidence from the molecular genetics study of the nAChR from PC12 cells [8] for the conservation of the amphipathic helix thought to contribute to the structure of the ion channel.

The similarities in the sensitivity of peripheral and central nAChR to H₁₂HTX contrasts with their differential sensitivities to competitive blockers. Thus the presynaptic nicotinic heteroreceptor modulating striatal dopamine release is insensitive to α -bungarotoxin, but is potently inhibited by neosurugotoxin [12] which in turn is ineffective at the neuromuscular junction [22]. Preliminary studies [13] suggested that the presynaptic autoreceptor on hippocampal nerve terminals may differ from its heteroreceptor counterpart in its sensitivity to α -bungarotoxin, but recent studies using a more sensitive perfusion schedule allowing the use of low concentrations of nicotine did not reproduce this observation and α -bungarotoxin at 0.1 μ M was largely without effect. Heterogeneity of brain nAChR is implicit in the cross-hybridization studies of Boulter et al. [9]. The present study demonstrates that two types of presynaptic nAChR are similar with respect to their sensitivity to H₁₂HTX and may share a common ionophore. This represents the first evidence for an HTX-sensitive ion channel associated with nAChR in the mammalian CNS, although such a mechanism is implicit in the characteristic fast depolarizations recorded in response to the iontophoresis of nicotinic drugs [7].

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