*Br. J. Pharmac.* (1977), **59**, 69–74

# THE PHARMACOLOGICAL PROPERTIES OF THE CHOLINERGIC FALSE TRANSMITTER, *N*-2-ACETOXYETHYL-*N*-METHYLPYRROLIDINIUM, AND ITS PRECURSOR, *N*-2-HYDROXYETHYL-*N*-METHYLPYRROLIDINIUM

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**1** The pharmacological properties of *N*-2-hydroxyethyl-*N*-methyl pyrrolidinium (pyrrolcholine) and its acetate ester, recently shown to be a false transmitter at the cholinergic electromotor synapses in *Torpedo marmorata*, also those of the corresponding morpholinium compounds (morpholinecholine, acetylmorpholinecholine), have been studied on the guinea-pig ileum, frog heart, frog rectus abdominis muscle, rat blood pressure, rat gastrocnemius muscle and dorsal muscle of the leech.

**2** Acetylpyrrolcholine and acetylmorpholinecholine are full cholinoceptor agonists with dose-response curves parallel to that of acetylcholine. They are, however, less potent. Acetylpyrrolcholine is relatively more potent as a muscarinic drug (molar potency about 30% of that of acetylcholine in the ileum but only 4% on the leech) whereas acetylmorpholinecholine is more strongly nicotinic. The unacetylated compounds are very weak agonists with potencies comparable to that of choline.

**3** Pyrrolcholine in high concentration showed a distinct neuromuscular blocking effect in the rat gastrocnemius muscle preparation. It is likely that this is a direct effect and not due to uptake by the presynaptic nerve terminals followed by conversion to a false transmitter since it was not reduced by hemicholinium-3, which is known to block uptake of choline and choline analogues by the presynaptic high affinity choline uptake system.

## Introduction

The concept of a 'false transmitter' is well established in the pharmacology of the sympathetic nervous system. Briefly, it is a substance which is taken up into adrenergic nerve terminals, and thence into synaptic vesicles, and on stimulation is released along with noradrenaline. Since many such substances are less active agonists than the natural transmitter, a partial blockade of synaptic transmission may result. The demonstration that only those substances which enter synaptic vesicles are released on stimulation has constituted important evidence for the 'vesicle hypothesis' in the adrenergic system (for review see Smith, 1972).

In principle, false transmitters should exist in the cholinergic system too, since it is unlikely that the storage mechanism in cholinergic terminals is completely specific for acetylcholine. However, the process by which acetylcholine is taken up into cholinergic synaptic vesicles is more complex and involves at least three steps: the entry of choline into the terminal via a high affinity choline permease (for references see Whittaker & Dowdall, 1975), the acetylation of choline to acetylcholine, a process known to be cytoplasmic in location (Fonnum, 1967) and the uptake of acetylcholine into the vesicles. The identification of false transmitters in the cholinergic system thus involves experiments with unesterified precursors, analogues of choline which are both taken up and acetylated.

Recent work has shown that the compound *N*-2-hydroxyethyl-*N*-methylpyrrolidinium (trivial name: pyrrolcholine) is a false transmitter precursor in the cholinergic electromotor system of *Torpedo* (Zimmermann & Dowdall, 1975a,b, 1976; Dowdall, Fox, Wächtler, Whittaker & Zimmermann, 1976) since the acetylated product is taken up into vesicles (as shown by subsequent isolation) and released on stimulation. Evidence for release from mammalian cholinergic endings is also forthcoming (Glick, Crane, Barker & Mittag, 1975; Collier, Barker & Mittag, 1976). A knowledge of the pharmacological properties of this compound and its acetate ester is thus of interest. Only a brief study of the acetate has so far appeared (Cho, Jenden & Lamb, 1972). In the study now to be described another putative false transmitter precursor *N*-2-hydroxyethyl-*N*-methyl morpholinium (trivial name: morpholinecholine) and its acetate ester have also been investigated for comparison.

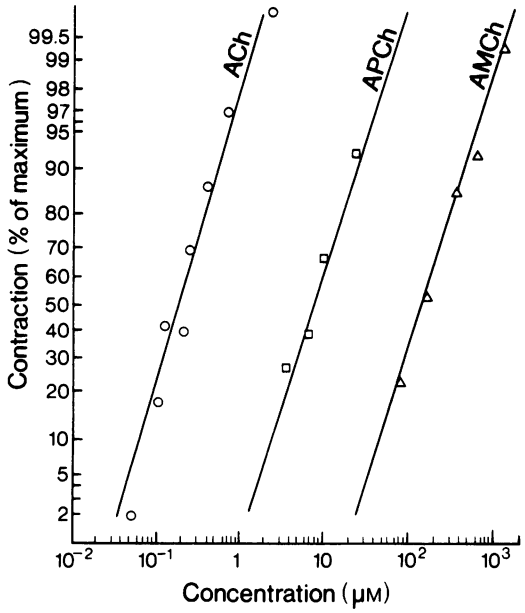


**Figure 1** Dose-response curves for (O) acetylcholine (ACh), (□) acetylpyrrolcholine (APCh) and (△) acetylmorpholinecholine (AMCh) applied to the dorsal muscle of the leech. Ordinates: percentage of maximum contraction plotted on a linear scale of probits; abscissae: logarithmic scale of doses.

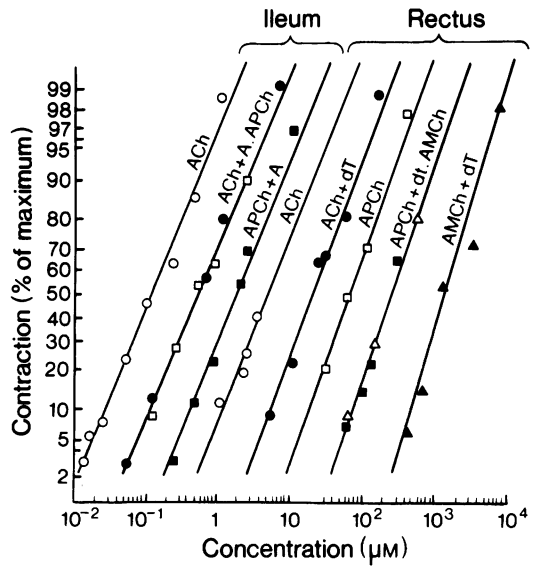


**Figure 2** Plots 1–3: effect of (filled symbols) atropine sulphate (A; 0.01 μM with respect to atropine) on the dose-response curves of (O) acetylcholine (ACh) and (□) acetylpyrrolcholine (APCh) applied to the guinea-pig ileum. Plots 4–8: effect of (filled symbols) (+)-tubocurarine chloride (dT; 0.1 μM with respect to base) on the dose-response curves of ACh, APCh and, in a different experiment, (△) acetylmorpholinecholine (AMCh) applied to the frog rectus abdominis muscle. Note that the blockade of the nicotinic effects of APCh and AMCh by dT and that of the muscarinic effect of APCh by A is about the same as that produced on ACh. Ordinates and abscissae as in Figure 1.

## Methods

### *Materials*

Pyrrolcholine and morpholinecholine were synthesized as iodides according to Barker & Mittag (1975); they were acetylated with acetylchloride according to Widlund & Heilbronn (1974) and the acetylation reaction was followed by the hydroxamate method (Hestrin, 1949). Acetylcholine perchlorate was used as a reference compound.

### *Pharmacological preparations*

The following preparations were used: leech dorsal muscle, frog rectus abdominis muscle, guinea-pig ileum (all as described by Whittaker & Barker, 1972), perfused frog heart, (Burn, 1952), rat blood pressure and sciatic nerve-gastrocnemius muscle preparation (Chiou, 1974).

For the last preparation Wistar rats (250–300 g) were anaesthetized with sodium pentobarbitone (35 mg/kg). The carotid artery was cannulated for blood pressure determination and the jugular vein for drug injection. The effect of the drugs on neuromuscular transmission was determined by recording isotonic contractions of the gastrocnemius muscle in response to supramaximal electrical stimulation once every 10 s through the sciatic nerve. Rectangular wave stimuli of 0.4 ms duration and 6–8 V strength were delivered by a stimulator through shielded platinum electrodes 4 mm apart placed on the nerve distally to a crushed region. The hypotensive responses of drugs were recorded simultaneously along with the neuromuscular effect. Dose-response curves were constructed for all substances and their $ED_{50}$ values were determined by probit plot analysis (Litchfield & Wilcoxon, 1949).

The $LD_{50}$ values of pyrrolcholine and acetylpyrrolcholine were determined in mice (20–25 g) according to Litchfield & Wilcoxon (1949). The drugs, dissolved in 0.9% w/v NaCl solution (saline), were given intraperitoneally in a volume of 0.1 ml.

## Results

The results obtained with the various preparations mentioned above are summarized in Table 1; in

columns 5–6 they are expressed as equipotent molar ratios and the figures are therefore inversely proportional to molar activities. Acetylpyrrolcholine and acetylmorpholinecholine are both full cholinoceptor agonists which reach an intrinsic activity of 1 (100% effect). Their dose-response curves are parallel to that of acetylcholine, and are shifted to the right (Figure 1), from which one may conclude that their affinity for the postsynaptic receptor is lower than that of acetylcholine. As can be seen from Table 1, acetylpyrrolcholine is a rather more potent muscarinic stimulant whereas acetylmorpholinecholine has a stronger effect at the nicotinic receptor. By contrast, the unacetylated choline analogues were very weak agonists with potencies comparable to that of choline.

The nicotinic effect of acetylpyrrolcholine and acetylmorpholinecholine is blocked by (+)-tubocurarine to approximately the same extent as acetylcholine. In all cases the dose-response curves are parallel and shifted to the right (Figure 2). The muscarinic effect of acetylpyrrolcholine can be blocked competitively by atropine (Figure 2).

As seen in Figure 3a,b the fall of blood pressure (lower tracings) in the rat is very short-lasting. Doses of acetylpyrrolcholine or acetylmorpholinecholine which halve the blood pressure produce only a very feeble blockade of neuromuscular transmission (upper traces). Only after pretreatment with atropine (0.25 mg/kg) and physostigmine (0.5 mg/kg; Figure 3c,d) is there a more marked reduction of the muscle contractions. This suggests that both esters are hydrolyzed by cholinesterases at about the same rate as acetylcholine. On the other hand, the precursors pyrrolcholine and morpholinecholine in high concentrations show a distinct neuromuscular blocking effect which is present without any pretreatment (Figure 4a,b). Morpholinecholine has no influence on the blood pressure whereas pyrrolcholine has a strongly pressor effect. In view of the very rapid response after drug injection it is unlikely that the effects observed are indirect and due to a false transmitter action. More likely what we are seeing is a direct reaction on the postsynaptic receptor. To test this further we repeated the experiments after pretreatment of the rat with hemicholinium-3 (1 mg/kg) to block the presynaptic uptake of the false transmitter precursor. Figure 5 shows that there is no failure of the pyrrolcholine effect on neuromuscular transmission as would be expected in the case of an indirect mechanism.

Preliminary studies of the toxicity of acetylpyrrolcholine and its precursor pyrrolcholine showed an $LD_{50}$, by intraperitoneal injection (95% confidence limits in parentheses), for acetylpyrrolcholine iodide, 76 mg/kg (65–87 mg/kg) and for pyrrolcholine iodide, 225 mg/kg (210–241 mg/kg). After administration of acetylpyrrolcholine the animals exhibited evidence of cholinoceptor stimulation (lacrimation, salivation and reduction of motility). Tremor was present only in some cases and then only very weakly.

## Discussion

In Table 2 the affinity of the two precursors for the synaptosomal high-affinity choline permease (as measured by their ability to inhibit choline uptake at low choline concentration) and their rate of acetylation by choline acetyltransferase are given to show that both compounds interact with the permease and are acetylated: however morpholinecholine is a poor substrate for choline acetyltransferase and a

**Table 1** Median effective doses ($ED_{50}$) and equipotent molar ratios, relative to acetylcholine, of acetylpyrrolcholine (APCh) and acetyl morpholinecholine (AMCh)

| | $ED_{50}$ (μM)* | | | Equipotent molar ratio (ACh = 1)* | |
|---|---|---|---|---|---|
| *Preparation, effect* | *Acetylcholine* | *APCh* | *AMCh* | *APCh* | *AMCh* |
| Leech muscle, contracture | 0.054 (8) | 1.5 (5) | 29 (2) | 28 | 760 |
| | (0.036–0.083) | (0.86–2.6) | (11–76) | (14–55) | (200–2900) |
| Frog rectus, contracture | 4.7 (3) | 70 (3) | 230 (3) | 15 | 280 |
| | (2.2–9.7) | (35–150) | (120–300) | (5–41) | (118–652) |
| Guinea-pig ileum, contracture | 0.14 (8) | 0.48 (4) | 1600 (4) | 3.6 | 7000 |
| | (0.08–0.23) | (0.22–1.1) | (670–2800) | (1.4–9.1) | (2300–21 000) |
| Frog heart, slowing and reduction of beat | 1.9 (4) | 5.4 (4) | 4500 (4) | 2.9 | 2400 |
| | (0.5–6.6) | (1.4–20) | (1000–20 000) | (0.7–13) | (440–12 700) |
| Rat blood pressure, fall† | 3.1 (4) | 7 (4) | 910 (2) | 2.9 | 395 |
| | (0.6–18.3) | (1.5–13) | (189–4390) | (0.4–21) | (51–3000) |

* Mean values: the figures in parentheses after the mean values are the no. of experiments, those below are the 95% confidence limits.
† Values are expressed as nmol/kg body weight.

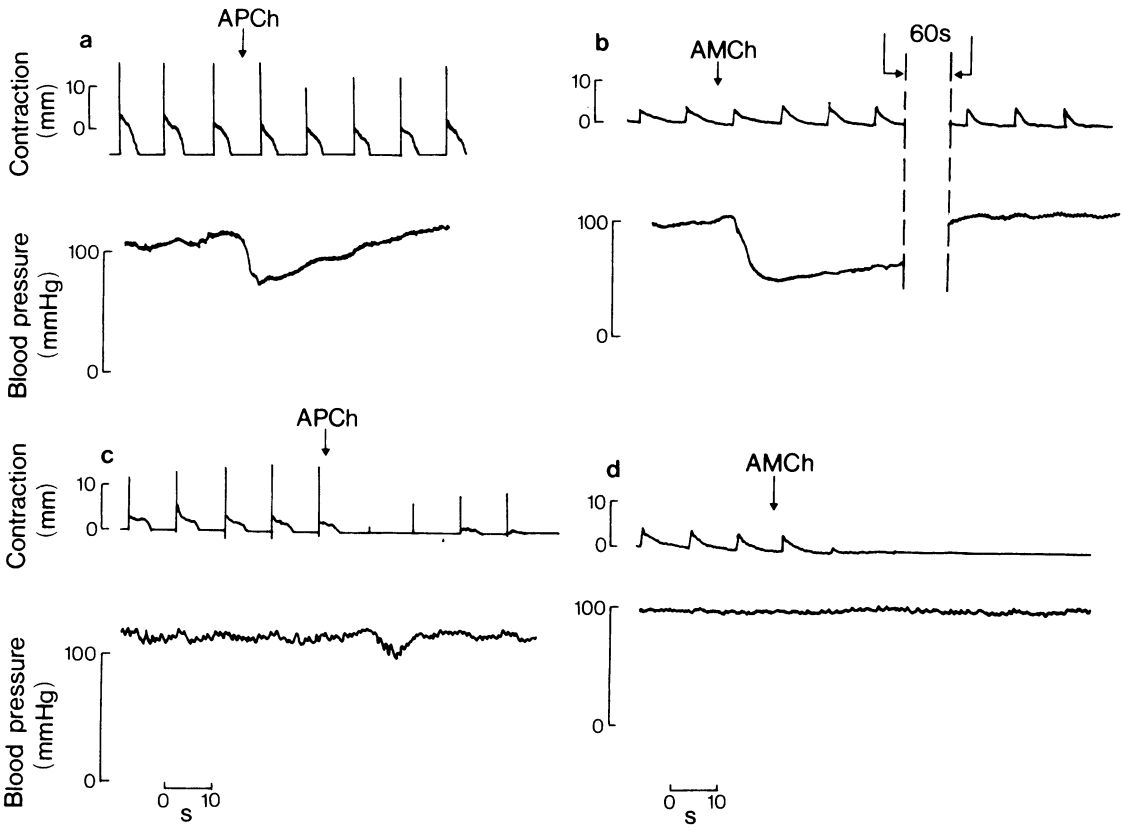


**Figure 3** (a) Effect of acetylpyrrolcholine (APCh; 15.5 μg/kg) on (upper trace contractions of the gastrocnemius muscle and (lower trace) blood pressure of the rat; (b) similar traces showing the effect of acetylmorpholinecholine (AMCh); (c, d) experiments similar to (a) and (b) except that the preparation was pretreated with atropine sulphate (0.25 mg/kg) and physostigmine sulphate (0.5 mg/kg).

**Table 2** The acetylation of *N*-hydroxyethyl-*N*-methyl-pyrrolidinium (PCh) and -morpholinium (MCh) methiodides by choline acetyltransferase and their inhibition of synaptosomal high-affinity choline permease

| *Source of preparation (Species, tissue)* | *Parameter (units)* | *PCh* | *MCh* | *Reference* |
|---|---|---|---|---|
| *Acetylation by choline acetyltransferase* | | | | |
| *Torpedo*, electric organ | $K_m$ (choline = 1) | 3.1 | 2.7 | M.J. Dowdall, unpublished |
| | $V_{max}$ (choline = 1) | 0.29 | 0.16 | |
| *Inhibition of synaptosomal permease* | | | | |
| Squid, optic lobe | $I_{50}$ (μM) at 1 μM choline | 6.8* | 100 | Barker, Dowdall & Mittag (1975) |
| Rat, striatum | | 3.7 | 37 | Simon, Mittag & Kuhar (1975) |
| *Torpedo*, electric organ | | 5.0 | 46 | M.J. Dowdall, unpublished |

* Calculated from $K_T$ and $V_{max}$ values for pyrrolcholine and choline

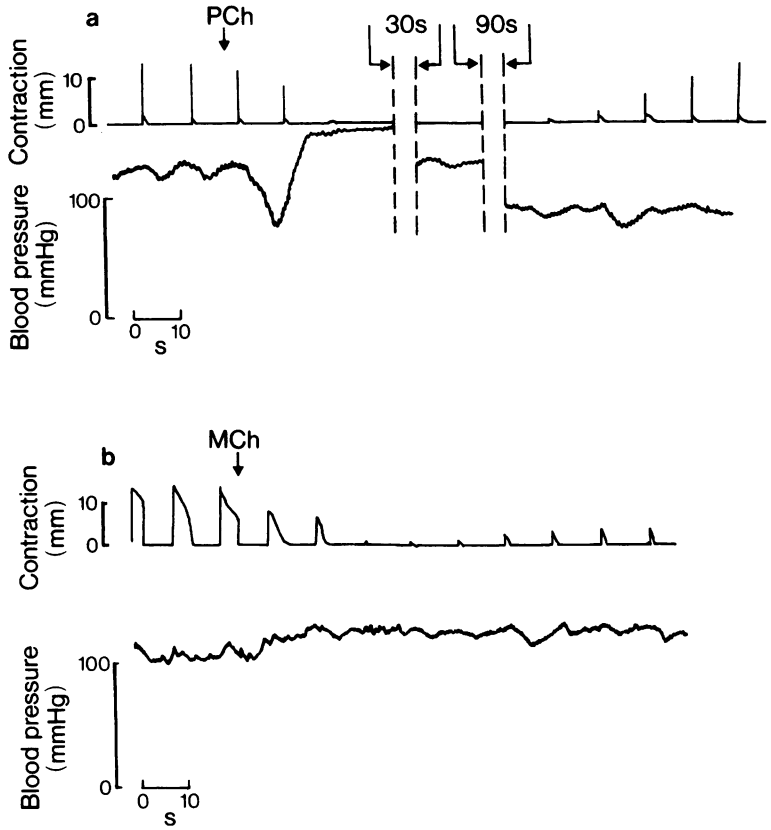


**Figure 4** Effect of (a) pyrrolcholine (PCh; 120 mg/kg) and (b) morpholinecholine (MCh; 128 mg/kg) on (upper traces) neuromuscular transmission and (lower traces) blood pressure in the rat. Note that in contrast to acetylpyrrolcholine, the effect of pyrrolcholine is to produce a sustained rise in blood pressure after an initial fall and both compounds show a more marked effect on neuromuscular transmission relative to their effect on blood pressure than (Figure 3) the acetylated compounds.

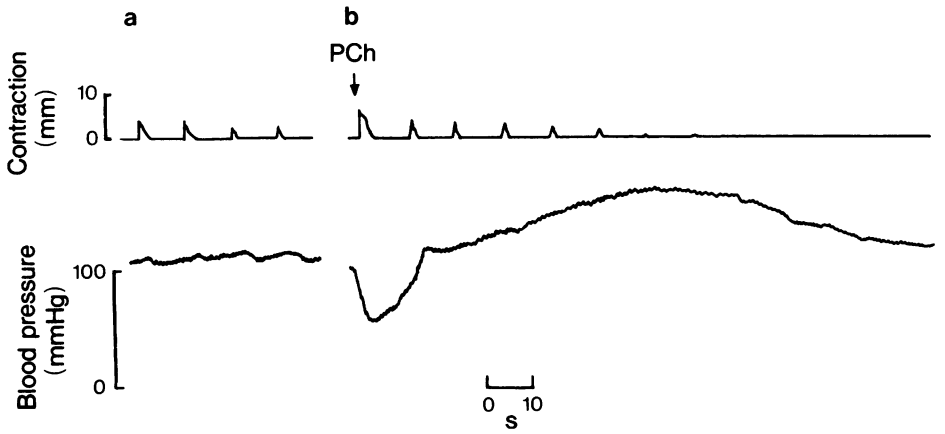


**Figure 5** (b) The effect of pyrrolcholine on (upper trace) neuromuscular transmission and (lower trace) blood pressure in the rat after injection of hemicholinium-3 bromide (1 mg/kg); (a) is a control trace before injection of pyrrolcholine. PCh = pyrrolcholine.

poor inhibitor of choline uptake; it is thus unlikely to compete effectively with choline for uptake and acetylation and its role as a false transmitter precursor has so far not been confirmed.

By contrast, pyrrolcholine is a somewhat better substrate for acetylation and is much more readily taken up into the terminals; in the *Torpedo* (Dowdall *et al.*, 1976) the acetylated product is known to be taken up into vesicles (by isolation of the vesicles and chromatographic identification of the ester) and to be released, along with acetylcholine, on stimulation, and there is evidence for release from mammalian cholinergic endings also (Glick *et al.*, 1975; Collier *et al.*, 1976). Since the acetylated product is considerably less potent as a cholinergic agonist than acetylcholine one might have expected pyrrolcholine to exert a synaptic blocking effect indirectly by way of conversion to a false transmitter. Although it did have a considerable blocking effect on the rat neuromuscular junction we were unable to demonstrate that this was other than a direct effect on the muscle cholinoceptors themselves. It remains to be seen whether effects consistent with a false transmitter precursor action would be uncovered at low doses and longer time intervals.

We are grateful to Dr. L.A. Barker for allowing us to refer to work in press and to Dr. M.J. Dowdall for providing compounds, for permitting us to include some of his unpublished results and for helpful discussions. The rat blood pressure and gastrocnemius experiments were done in the Pharmakologisches Institut of Göttingen University, by kind permission of Professor G. Schmidt.

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*(Received April 20, 1976. Revised July 22, 1976)*