J. Pharm. Pharmac., 1970, **22**, 433–440 *Received November* 17, 1969

Pharmacological actions of phosphocholine 2,6-xylyl ether bromide (phospho-TM10; PTM10)

I. E. HUGHES

Department of Pharmacology, The Medical School, Thoresby Place, Leeds, U.K.

PPP-Trimethyl-2-(2,6-xylyloxy)ethylphosphonium bromide (PTM10; phospho-TM10), the phosphorus analogue of xylocholine (TM10), has been found to possess local anaesthetic, α-adrenoceptor and ganglion blocking properties. It also shows a blocking action at the neuromuscular junction but has no detectable adrenergic neuron blocking properties. The relation of these results to the 'cholinergic link" hypothesis of Burn and Rand is discussed.

It is well known that substitution of phosphorus for nitrogen in compounds which show agonist activity at acetylcholine receptors results in a reduction in potency (Hunt & Renshaw, 1925; Holton & Ing, 1949). Since Burn & Rand (1959) have postulated that acetylcholine is involved in the release of noradrenaline from adrenergic nerve terminals and that adrenergic neuron blocking agents, such as bretylium and xylocholine, act by preventing this "nicotinic" action of acetylcholine on the noradrenaline stores (Burn & Rand, 1962), it was felt that it might be of interest to investigate the pharmacology of *PPP*-trimethyl-2-(2,6-xylyloxy)ethylphosphonium bromide (phospho-TM10; PTM10) with special reference to any action on the adrenergic nerve or at "nicotinic" sites of action of acetylcholine. The synthesis and an initial pharmacological investigation of phospho-TM10 (PTM10) have already been reported (Clark & Hughes, 1970) and a more comprehensive pharmacological assessment of this compound is now presented.

EXPERIMENTAL

Rat blood pressure

Male rats, 180–250 g, were anaesthetized with pentobarbitone (50 mg/kg) intraperitoneally, or with urethane (15% w/v in 0·9% NaCl; 1·2 g/kg) by the same route. Plastic tracheal, left carotid and right jugular cannulae were inserted and each animal received 100 units of heparin when the operative procedure was complete. All rats were artificially respired using a Palmer Miniature Ideal pump set to deliver 2·5 ml of air at a rate of 100/min. Blood pressure was recorded from the carotid artery on a Devices M2R recorder with a Bell and Howell pressure transducer (Type 4-327-L221) using a Devices preamplifier. Heart rate was determined by counting the number of pulse waves in a 5 s period. All injections were made through the jugular cannula unless otherwise stated and were washed in with 0·1 ml 0·9% NaCl. Rats were pithed under hexobarbitone anaesthesia (250 mg/kg administered intraperitoneally) using the approach through the orbit. These rats received 1·5 mg/kg atropine sulphate intraperitoneally 30 min before the anaesthetic was administered.

Cat blood pressure and nictitating membrane

Anaesthesia was induced in cats of either sex, 1·3–2·5 kg, with ether and was maintained with chloralose (80 mg/kg) injected through a cannula in the right femoral vein. The trachea was cannulated and respiration maintained artificially. The right postganglionic and left preganglionic cervical sympathetic nerves were exposed and stimulated electrically for 15 s as required with rectilinear pulses of 0·5 ms duration and supramaximal voltage delivered at a rate of 20 or 50 shocks/s. The right vagus was exposed and stimulated electrically as required. All stimuli were applied through hook electrodes from a Palmer H44 stimulator and all nerves were sectioned proximal to the stimulating electrodes and covered with cotton wool soaked in saline-equilibrated liquid paraffin. Contractions of the nictitating membranes were recorded with isometric transducers (Devices, Type 2STO2) and blood pressure was measured from the right femoral artery as described for the rat. Heart rate was obtained from the pulse wave recorded by the pressure transducer using a Devices DC3 preamplifier and Model B Instantaneous Ratemeter. All recordings were made on a Devices M4 recorder and drugs were dissolved in saline, injected through the femoral cannula and washed in with 0·5 ml 0·9% NaCl. All animals received 1000 units of heparin when the operative procedure was complete.

Intracutaneous weal test

As described by Bülbring & Wajda (1945).

Isolated cervical vagus nerve

As described by Clark & Hughes (1966) except that stimuli were applied from a Grass S8 stimulator through a Grass isolation unit (Type SIU 4678). Recordings were made differentially through a Tektronix Type 122 preamplifier and were photographed after display on a Tektronix Type 502A oscillograph. The nerve under one of the recording electrodes was destroyed by crushing.

Phrenic nerve-diaphragm

Preparations were mounted on Palmer phrenic nerve electrodes in Krebs solution at 37° and gassed with 5% carbon dioxide in oxygen. Stimuli were applied either directly or indirectly from a Palmer H44 stimulator and recordings were made on a Devices M2R recorder with a Devices isometric transducer.

Frog rectus abdominis

Preparations were removed from freshly killed frogs, set up in Frog Ringer at room temperature and gassed with air. Recordings were made and displayed as for the phrenic nerve-diaphragm.

The drugs used were: acetylcholine bromide, atropine sulphate monohydrate, carbachol chloride, chloralose, heparin injection B.P., hexabarbitone sodium, hexamethonium bromide, (−)noradrenaline bitartrate monohydrate, pentobarbitone sodium, piperoxan hydrochloride, phentolamine methane sulphonate, neostigmine methyl sulphate, tetraethylammonium iodide, (+)tubocurarine chloride, and urethane. *NNN*-trimethyl-2-(2,6-xylyloxy)ethylammonium bromide (xylocholine; TM10), *NNN*-trimethyl-2-(phenoxy)ethylammonium bromide (TM1), and *PPP*-trimethyl-2-(2,6-xylyloxy)ethylphosphonium bromide (phospho-TM10; PTM10) were

synthesized in these laboratories. All doses and concentrations are expressed in terms of these salts.

RESULTS

Action on the cardiovascular system of the rat

Administration of PTM10 (2–15 mg/kg) to rats anaesthetized with urethane or pentobarbitone produced an immediate bradycardia lasting some 5–20 min which was accompanied by a large depressor response lasting 20–60 min. The depressor response was preceded by a shortlived pressor response and the magnitude and duration of the depressor response and bradycardia were related to the dose of PTM10

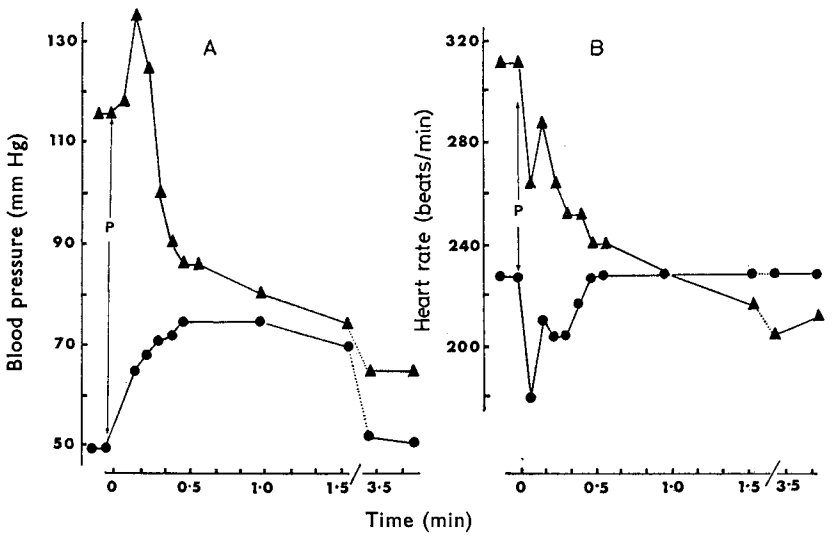

FIG. 1. The effects of PTM10 (1·5 mg, intravenously at P) on the blood pressure (A) and heart rate (B) of a pentobarbitone anaesthetized rat (▲, weight 195 g) and an atropinized pithed rat (●, weight 220 g). Time (min) after the administration of PTM10 is plotted on the abscissa.

administered (Fig. 1). Administration of a second dose of PTM10 during the depressor phase following an initial high dose failed to produce any further reduction in the blood pressure though the initial pressor response was still seen.

In the pithed atropinized rat, similar doses of PTM10 failed to produce a depressor response and the bradycardia was less marked and of much shorter duration than that seen in the anaesthetized animal. The initial pressor response was still seen however and was augmented in both magnitude and duration (Fig. 1). This pressor response was produced on repeated administration of PTM10 and was not reduced by acute bilateral adrenalectomy or by treatment of the rats with α-blocking agents (phentolamine, 0·5 mg/kg; piperoxan, 4 mg/kg) administered intravenously 5 min before the dose of PTM10. Hexamethonium (1·5 mg/kg) also failed to reduce the pressor response to PTM10.

In pithed atropinized rats, the pressor responses evoked by administration of noradrenaline and by the ganglion stimulant TM1 were antagonized by similar doses of PTM10, the action against noradrenaline being shorter in duration than the action against TM1 (Fig. 2).

FIG. 2. Pithed atropinized rat (weight 240 g). The effects of noradrenaline (0·05 μg intravenously at ●) and TM1 (20 μg intravenously at ■) before and after 0·8 mg PTM10 intravenously at ▲ on the blood pressure in mm Hg. Time marker, 60 s.

Action on cat blood pressure and nictitating membranes

In doses of 1–8 mg/kg, PTM10 caused a fall in blood pressure and heart rate in the anaesthetized cat. The bradycardia was less marked in this species than in the rat and the initial pressor response which was always seen in the rat was observed in only one out of 10 experiments (Fig. 3).

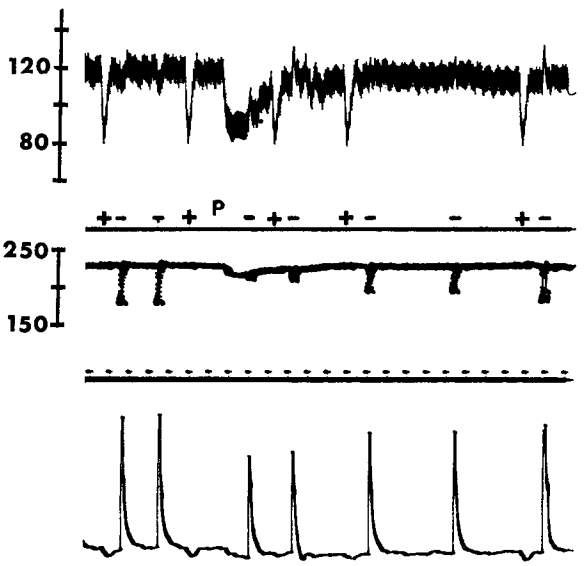

FIG. 3. Nictitating membrane preparation of the cat (1·4 kg). Upper record: Blood pressure in mm Hg. Middle record: Heart rate in beats/min. Lower record: contraction of the nictitating membrane. At -, electrical stimulation was applied to the vagus (10/s, 0·2 ms duration, 10 V for 7 s) and preganglionic cervical sympathetic (20/s, 0·5 ms duration, 10 V for 15 s) nerves simultaneously. At P, 4·5 mg PTM10 and at +, 0·5 μg acetylcholine were injected intravenously. PTM10 reduced the response of the nictitating membrane and the fall in heart rate produced by electrical stimulation with a similar time course but left the response to injected acetylcholine unchanged. Time marker, 60 s.

The fall in heart rate produced by electrical stimulation of the distal portion of the sectioned vagus was reduced or abolished by these doses of PTM10, though the depressor response to injected acetylcholine remained unchanged. Responses of the nictitating membrane to stimulation of the cervical sympathetic nerve preganglionically were also reduced, the time course of this reduction being similar to that of the effect on vagal stimulation (Fig. 3). Responses of the nictitating membrane to postganglionic stimulation were transiently reduced, this effect being smaller and of

much shorter duration than the effect against preganglionic stimulation. In all cases the blocking action of PTM10 was fast in onset and of only moderate duration, the responses returning to control levels within 40 min of the administration of the dose of PTM10. Although larger doses killed the animals (producing a precipitous fall in heart rate, pulse pressure and blood pressure which was quickly followed by the death of the animal), the responses of the nictitating membranes to pre- and post ganglionic stimulation of the cervical sympathetic nerves returned to control levels within 45 min after the administration of 50 mg/kg of PTM10 in divided doses over a period of 1–2 h in three experiments, and after administration of 100 mg/kg over 3 h in one further experiment (Fig. 4).

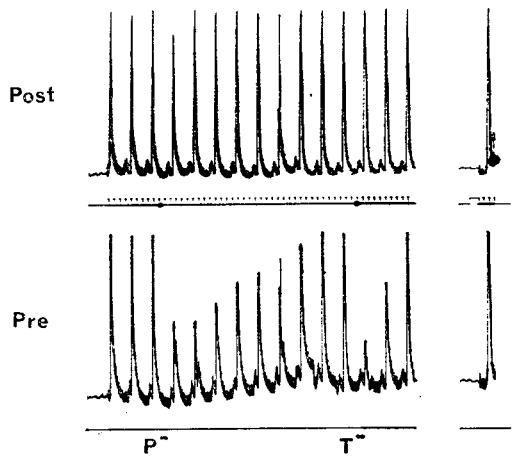

FIG. 4. Nictitating membrane preparation of cat (1·9 kg). Responses are to stimulation (0·5 ms duration, 50/s, 14 V: applied for 15 s every 3 min 45 s for the whole experiment) of the pre-(lower) and post-(upper) ganglionic cervical sympathetic nerves. At P, 9 mg PTM10 and at T, 17 mg tetraethylammonium were injected intravenously. Time interval between parts 1 and 2 of the record was 160 min and during the first 120 min of this period 50 mg/kg PTM10 was administered in divided doses and had no prolonged effect on the response to electrical stimulation. Time marker, 60 s.

Assessment of local anaesthetic activity

Intracutaneous injections of solutions of PTM10 in 0·9% NaCl into guinea-pigs according to the method of Bülbring & Wajda (1945) produced a loss of sensation in the area injected. A delay in the onset of the anaesthesia, which is characteristic of xylocholine, was also apparent with PTM10, though to a lesser extent. Since differences in rates of onset of anaesthesia complicate estimations of potency by this method, no accurate comparisons of potency were made. However, solutions of xylocholine and PTM10 of equal molarity produced approximately similar degrees of anaesthesia indicating that there was no great difference in the local anaesthetic potency of these two compounds.

In the isolated cervical vagus preparation of the rabbit, PTM10 in concentrations of 0·5–1 mg/ml produced a reduction in the size of the action potential which was usually in excess of 90% after 15 min exposure to the drug. In similar concentrations xylocholine also reduced the size of the action potential though the effect was not as great as that seen with PTM10. With both drugs complete abolition of the action potential could be achieved on exposure to higher concentrations and complete

reversal of this blockade was usually produced after washing the preparation for 2–4 h (Fig. 5).

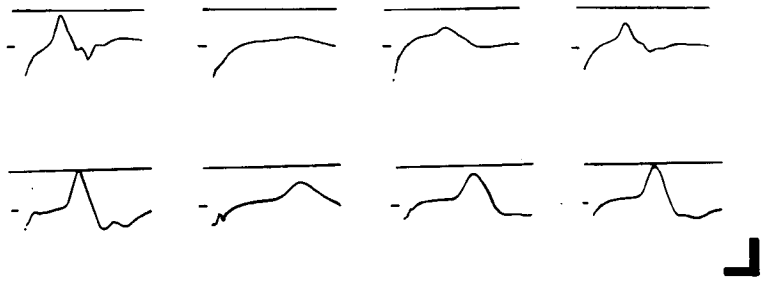

FIG. 5. Action of PTM10 (0·6 mg/ml—upper record) and xylocholine (0·6 mg/ml—lower record) in reducing the size of the action potential recorded on stimulation (0·5 ms duration, 32 V) of the isolated cervical vagus from the rabbit. From left to right: control: after 15 min exposure to the drug: after washing for 1 and 3 h respectively. Calibration at bottom right is 3 mV vertical and 20 ms horizontal.

Action on the isolated phrenic nerve-diaphragm

In concentrations of 10 μg/ml and above, PTM10 produced a reduction in the twitch tension developed in the isolated diaphragm in response to electrical stimulation of the phrenic nerve. At low concentrations development of the blockade was slow and equilibrium was not attained in 90 min when the experiments were terminated. At higher concentrations the blockade was fast in onset and responses to direct stimulation of the muscle were unaffected by concentrations of PTM10 which completely abolished the response to indirect stimulation. The blockade could be reversed easily on washing the tissue and was partially reversed by KCl (0·4 mg/ml) but not by tetraethylammonium (50 μg/ml) or by neostigmine (5 μg/ml), though these concentrations were effective in reversing the blockade due to tubocurarine. Partial blocking concentrations of tubocurarine and PTM10 were additive and in no case was any antagonism observed. Estimations of blocking potency after exposure of the tissue to the drug for 15 min (i.e. under non-equilibrium conditions) indicated that PTM10 was a more potent blocking agent than xylocholine on this preparation, concentrations needed to produce 50% blockade being 35 and 45 μg/ml for PTM10 and 52 and 60 μg/ml for xylocholine in two experiments respectively (Fig. 6).

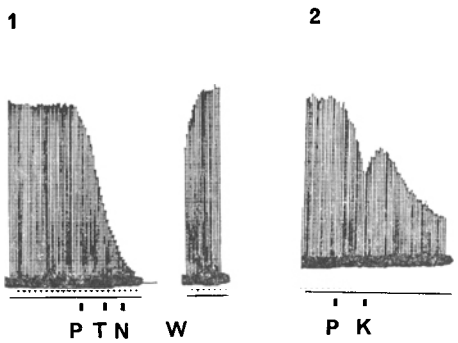

FIG. 6. Rat phrenic nerve-diaphragm. Maximal twitches in response to stimulation of the phrenic nerve (0·03 ms duration, 11 V, 3/min). Time marker, 60 s. Part 1. Effect of PTM10 (80 μg/ml at P) and of tetraethylammonium (50 μg/ml at T) and neostigmine (5 μg/ml at N). Time interval between the two sections of part 1 was 45 s during which the tissue was washed once by drainage. Part 2. Effect of KCl (0·4 mg/ml at K) on the blockade produced by PTM10 (75 μg/ml at P).

Action on the rectus abdominis

On this preparation responses to both acetylcholine and carbachol were antagonized by PTM10 in concentrations above 2 μg/ml. Dose-response curves constructed in the presence of various concentrations of PTM10 showed that not only were the dose-response curves to acetylcholine and carbachol shifted to higher concentrations along the dose axis, but that the maximal response which could be elicited from the tissue was also reduced. (Fig. 7).

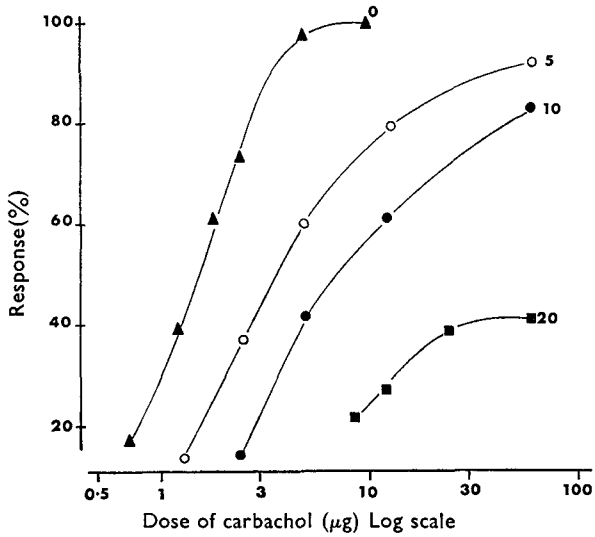

FIG. 7. Frog rectus abdominis. Plot of $\log_{10}$ (in μg) against response (% of maximal) for carbachol in the presence of 0, 5, 10 and 20 μg/ml of PTM10.

DISCUSSION

Clark & Hughes, (1970) indicated that replacement of the nitrogen atom in xylocholine by phosphorus produced a compound (PTM10) which was devoid of adrenergic neuron blocking properties. The results here presented substantiate this finding.

Since PTM10 reduced the effect of noradrenaline on the blood pressure of the pithed rat and also had a more prolonged blocking action against the ganglion stimulant TM1, it is apparent that it possesses α-blocking properties and also interferes with transmission in adrenergic nerves. As the response of the nictitating membranes to preganglionic stimulation was much reduced while the response to postganglionic stimulation remained relatively unchanged it is probable that this interference takes place at the level of the ganglion.

The effect of PTM10 on vagal stimulation is unlikely to be due to any antimuscarinic action since the response to injected acetylcholine was unchanged. It is likely therefore that this action is also due to ganglion blockade and this is supported by the observation that the time course of the effect is similar to that of the action of PTM10 on the response of the nictitating membrane to electrical stimulation of the cervical sympathetic nerve preganglionically.

The possession of ganglion blocking properties by PTM10 could also explain the effect of this compound on the blood pressure and heart rate of the cat and rat, the differences in size of the reduction in heart rate being due to differences in the degree of normal sympathetic tone in the two species. The initial pressor response to

PTM10, seen mainly in the rat, was probably not due to an action at ganglia as it was unaffected by hexamethonium. Release of catecholamines (which is responsible for the pressor action of xylocholine) also seems unlikely in the case of PTM10 as bilateral adrenalectomy and α-blocking agents did not reduce the response. The mechanism of this initial pressor response has yet to be established but may be due to a direct action.

The blocking actions of PTM10 were fast in onset and of only moderate duration. In no case, even at high dose levels, was any blocking action observed which had the characteristic long duration of the adrenergic neuron blocking agents.

The actions of PTM10 in the guinea-pig intracutaneous weal test and on the isolated vagus can be accounted for in terms of local anaesthetic activity and in this respect PTM10 appears to be equipotent or slightly more potent than xylocholine. The local anaesthetic properties demonstrated in these preparations probably account for the blocking action of PTM10 on the Finkleman preparation which was demonstrated earlier (Clark & Hughes, 1970).

The actions of PTM10 on the phrenic nerve diaphragm preparation appear to be similar to those of bretylium (Dixit, Gulati & Gokhale, 1961). Blockade by PTM10 was additive to a partial tubocurarine blockade, was easily reversed by washing and by KCl but was unaffected by neostigmine and tetraethylammonium. This blocking action was at least as potent as that possessed by xylocholine. In contrast to bretylium however, PTM10 had a non-competitive blocking action against acetylcholine and carbachol on the frog rectus abdominis muscle.

The multiple actions of PTM10 make it difficult to establish that this compound has no adrenergic neuron blocking properties. However, the fact that all the actions of PTM10 that I observed can be accounted for by mechanisms other than adrenergic neuron blockade, suggests that any adrenergic neuron blocking properties possessed by PTM10 must be very weak. In spite of this, the actions of PTM10 at the neuromuscular junction are at least as strong as those of xylocholine and are of a similar type to those possessed by other adrenergic neuron blocking agents. It may be therefore that support for the Burn & Rand "cholinergic link" hypothesis drawn from the actions of drugs at the neuromuscular junction should be treated with caution.

The lack of adrenergic neuron blocking activity in PTM10 indicates that the requirements for adrenergic neuron blocking activity are very closely defined in terms of molecular structure. The loss of adrenergic neuron blocking properties on substitution of phosphorus for nitrogen in xylocholine is not dissimilar to the reduced potency observed in phosphonium analogues of compounds which are active at acetylcholine receptors and might therefore be interpreted as support for the Burn & Rand "cholinergic link" theory of transmission at adrenergic nerve terminals.

REFERENCES

BÜLBRING, E. & WAJDA, I. (1945). *J. Pharmac. exp. Ther.*, **85**, 78–84.
BURN, J. H. & RAND, M. J. (1959). *Nature, Lond.*, **184**, 163–165.
BURN, J. H. & RAND, M. J. (1962). *Advances in Pharmacology*, **1**, 1–30.
CLARK, E. R. & HUGHES, I. E. (1966). *Br. J. Pharmac. Chemother.*, **28**, 105–128.
CLARK, E. R. & HUGHES, I. E. (1970). *J. Pharm. Pharmac.*, **22**, 279–283.
DIXIT, B. N., GULATI, O. D. & GOKHALE, S. D. (1961). *Br. J. Pharmac. Chemother.*, **17**, 372–379.
HOLTON, P. & ING, H. R. (1949). *Ibid.*, **4**, 190–196.
HUNT, R. & RENSHAW, R. R. (1925). *J. Pharmac. exp. Ther.*, **25**, 315–355.