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SYNTHESIS AND PHARMACOLOGY OF NICOTINYLCHOLINE AND THREE BISQUATERNARY RELATED DERIVATIVES

B. HOLMSTEDT,* L. LARSSON† and A. SUNDWALL†

*Department of Pharmacology, Karolinska Institutet, Stockholm 60, Sweden
and
†Research Institute of National Defence, Dept. 1, Sundbyberg 4, Sweden

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Abstract—Nicotinylcholine and three related bisquaternary derivatives, nicotinylcholine iodide methiodide, nicotinylhomocholine iodide methiodide and pyridyl-3-acetycholine iodide methiodide, have been synthesized and studied pharmacologically. Nicotinylcholine is in general less potent than naturally-occurring choline esters. It is practically devoid of muscarinic actions, but in cats in doses of 5–10 mg/kg body weight it possesses pronounced nicotinic actions (ganglion-stimulating and neuromuscular-blocking actions). It is split by butyrocholinesterase at approximately the same rate as acetylcholine. On the frog rectus nicotinylcholine is about 300 times less potent than acetylcholine.

The corresponding bisquaternary compound, nicotinylcholine iodide methiodide, is not split by cholinesterases, and in doses of 10–20 mg/kg body weight it possesses ganglion-blocking properties. It has no effect on neuromuscular transmission as revealed by the cat sciatic nerve–gastrocnemius preparation. The other two bisquaternary compounds, nicotinylhomocholine iodide methiodide and pyridyl 3-acetylcholine iodide methiodide, have neuromuscular blocking actions in doses of 5–10 mg/kg body weight, but have little effect on the cat blood pressure. They are not split by cholinesterases.

ALIPHATIC choline esters have been studied in great detail for many years.[1, 2] The growing number of naturally-occurring esters of this type, both saturated[3, 4] and unsaturated,[5, 6] have prompted the study of numerous synthetic analogues. Among the naturally-occurring choline esters, so far only one compound containing a cyclic component has been found, urocanylcholine (murexine).[7]

In spite of the fact that nicotine has played such an important role in the study of the nervous system only scanty data are available concerning the choline ester of nicotinic acid, nicotinylcholine.[8] This paper deals with the synthesis and pharmacological effects of nicotinylcholine and some quaternary analogues, since the quaternization at the heterocyclic nitrogen might be expected to result in drugs with interesting pharmacological properties.

EXPERIMENTAL

Preparation of the esters

Nicotinylcholine iodide. A solution of 0·31 mole of 2-dimethylaminoethanol in 50 ml of benzene was added to 0·10 mole of crude nicotinyl chloride in 75 ml of benzene. The latter reagent was obtained from potassium nicotinate and oxalyl

chloride according to Wingfield *et al.*[9] The reaction mixture was refluxed for 4 hr. The dimethylaminoethanol hydrochloride formed was separated by filtration, and the filtrate was evaporated *in vacuo*. A viscous oil was obtained which was washed with 10 ml of cold water, dissolved in ether and dried with anhydrous sodium sulphate. After removal of the ether, 2-dimethylaminoethyl nicotinate was distilled, b.p. 90 °C/0·10 mm Hg. Yield 40 per cent, calculated on nicotinic acid.

2-Dimethylaminoethyl nicotinate (0·10 mole) was dissolved in 130 ml of anhydrous ether and 0·10 mole of methyl iodide was added. The reaction mixture was kept overnight at room temperature and yellow crystals of nicotinylcholine iodide were filtered off, washed with anhydrous ether and recrystallized from absolute ethanol. That quaternization of the ester had taken place at the nitrogen of the ester group and not at the pyridine nitrogen was shown by the isolation of choline as the reineckate after hydrolysis, as follows.

The quaternized ester (0·1017 g) was dissolved in 50 ml of water; 20 ml of this solution were hydrolysed by means of 20 ml of a 10% trisodium phosphate solution. To the hydrolysate 10 ml of a freshly-prepared 2% reineckate solution were added, and after 2 hr the precipitate was filtered off, washed with 10 ml of cold water and dried to constant weight at 110 °C. Found: 0·0510 g. Calc. for choline reineckate: 0·0511 g. Choline chloride (0·1000 g) treated in the same manner gave 0·1217 g choline reineckate against calculated 0·1211 g, while the hydrolysed methiodide of m ethy nicotinate gave no precipitation with the reineckate solution of the pH in question.

Nicotinylcholine iodide methiodide. 2-Dimethylaminoethyl nicotinate (0·10 mole) was dissolved in 200 ml of absolute methanol and 0·21 mole of methyl iodide was added. After 5 days the yellow crystals were filtered off and washed with anhydrous ether.

Nicotinylhomocholine iodide methiodide. 3-Dimethylaminopropyl nicotinate was prepared from nicotinyl chloride and 3-dimethylaminopropanol[10] in the same way as the ethyl ester: b.p. 95·5–97 °C/0·10 mm Hg, yield 61 per cent. The ester (0·10 mole) was dissolved in 200 ml of absolute ethanol, and the solution was refluxed for 3 hr with 0·21 mole of methyl iodide. After 6 days the yellow crystals were filtered off and washed with warm ethanol.

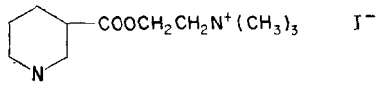
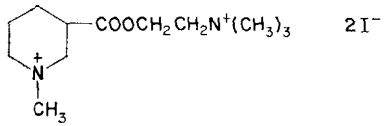
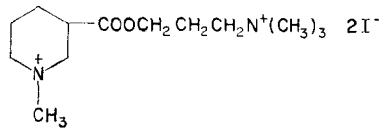
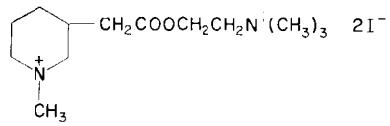
Pyridyl-3-acetylcholine iodide methiodide. 2-Dimethylaminoethyl pyridyl-3-acetate was synthesized in the same way as the corresponding nicotinate from pyridyl-3-acetic acid[11] and 2-diemethylaminoethanol. The viscous oil obtained was not purified by distillation, because it showed a tendency to decompose. The yield of the undistilled ester was 62 per cent. The crude ester (0·10 mole) dissolved in 400 ml of absolute ethanol was refluxed for 3 hr with 0·22 mole of methyl iodide and the crystals formed after 6 days were filtered off and washed with warm ethanol.

The formulae and analytical data of the compounds synthesized are given in Table 1.

Pharmacological and biochemical methods

Recording of respiration, blood pressure and muscular contractions. The effects of the esters were studied in ten anaesthetized cats. Anaesthesia was induced with ether followed by allobarbitone (50 mg/kg body weight) and was maintained by further

TABLE 1. ANALYTICAL DATA

Compound	Formula	Molecular weight	% C Calc.	% C Found	% H Calc.	% H Found	% I Calc.	% I Found	Yield in % calculated on the acid
Nicotinylcholine iodide (L 7)		336·2	39·3	39·1	5·1	5·0	37·7	37·7	34
Nicotinylcholine iodide methiodide (L 3)		478·1	30·1	30·2	4·2	4·2	53·1	52·7	20
Nicotinylhomocholine iodide methiodide (L 42)		492·2	31·7	31·5	4·5	4·3	51·6	51·8	37
Pyridyl-3-acetylcholine iodide methiodide (L 30)		492·2	31·7	32·3	4·5	4·3	51·6	49·0	14

small doses of allobarbitone. Drugs were injected intravenously through a plastic cannula in the femoral vein. Respiration, blood pressure, and contractions of the gastrocnemius muscle and of the nictitating membrane were recorded by passing the output of suitably arranged transducers into a Grass Model 5 Polygraph. Respiration was recorded as pressure differences in the tracheal cannula by means of a pressure transducer (Model PT 5, Grass Instrument Corporation). Blood pressure was recorded by means of a Statham electromanometer from the left carotid artery.

The effects of drugs on neuromuscular transmission were determined by recording semi-isometric contractions of the gastrocnemius muscle in response to supramaximal electrical stimulation of the sciatic nerve. A force displacement transducer Model FT 10, Grass Instrument Corporation, was used for recording the muscle contractions and the electrical stimuli were applied to the nerve distally to a crushed region by means of a Grass stimulator, Model S4, and shielded silver electrodes.

The neuromuscular blocking effect of the drugs is expressed in terms of the PD_{50} (namely, the dose required to produce 50 per cent blockade) and of the duration of such a block. To determine these quantities, the drugs were injected intra-arterially through a cannula in the femoral artery of the leg opposite to that being stimulated, the tip of the cannula lying proximally to the aortic bifurcation. The percentage diminution of twitch height and duration of block were recorded for three doses and the PD_{50} and the corresponding duration estimated by graphical interpolation. Succinylcholine has been included for comparison. Contractions of the nictitating membrane were recorded by means of force-displacement transducer FT 03. The cervical sympathetic trunk was exposed and dissected from the vagus. The excitability of the membrane was tested by stimulating the preganglionic trunk for 10–15 sec with supramaximal rectangular pulses of duration of 0·5 msec and frequency of 20 c/s before and after administration of drugs.

Frog rectus abdominis muscle preparations. The procedure was essentially that of Chang and Gaddum.[12] In this and the following preparations the activity of the esters was expressed as the relative molar potency in percentage units with acetylcholine or succinylcholine as standards.

Guinea-pig ileum preparations. A test bath of 6 ml volume was used. An automatic timer regulated the cycle of operations except for the addition of the compounds which was made manually in a small volume on top of the test bath.

Rat phrenic nerve–diaphragm preparation. The procedure was that described by Bülbring.[13] A test bath of 18 ml volume was used. The drug was left in contact with the preparation for 3 min. The nerve was stimulated with supramaximal single shocks, 10 per min of 0·2 msec duration.

Hydrolysis of esters by cholinesterases. This was measured electrometrically at 25 °C and pH 8 in buffer as described by Tammelin.[14] The following preparations were used: purified human plasma cholinesterase stated to correspond to Cohn's plasma fraction IV-6-3 (AB Kabi), human red cell cholinesterase prepared by haemolysing red cells with distilled water. Serum cholinesterase was inactivated by heating for

30 min at 70 °C, red cell cholinesterase by using a selective inhibitor, 1 : 5-bis (4-*N*-allyl-*N*-methylaminophenyl)-pentanone-3 dimethobromide (B.W. 284C51),[15] at a final concentration of 1×10^{-3} M. The substrate concentrations were: acetylcholine, 25 mM (human plasma cholinesterase) or 8 mM (human red cell cholinesterase), other esters 8 mM. Enzyme concentrations were adjusted to give convenient rates of hydrolysis with the different substrates. The rate of hydrolysis was expressed as μmoles acid liberated per ml per min.

RESULTS

Assay of the new esters on conventional test organs like the frog rectus and the guinea-pig ileum revealed that the nicotinylcholines had a very feeble action in comparison with acetylcholine. The nicotinic action (frog rectus) was, however, considerably stronger than the muscarine-like action (guinea-pig ileum) which on the whole may be said to be lacking (Table 2).

TABLE 2. ACTIVITY OF NICOTINYLCHOLINE AND THREE QUATERNARY ANALOGUES ON FROG RECTUS ABDOMINIS MUSCLE AND GUINEA-PIG ILEUM

Ester	Relative molar potency (acetylcholine = 100)	
	Frog rectus	Guinea-pig ileum
Nicotinylcholine iodide	0·33	<0·002
Pyridyl-3-acetylcholine iodide methiodide	0·14	<0·002
Nicotinylhomocholine iodide methiodide	<0·05	<0·002
Nicotinylcholine iodide methiodide	<0·05	<0·002

Hydrolysis by cholinesterases

All the esters synthesized were tested for their hydrolysis by cholinesterase, which was compared with that of acetylcholine under identical conditions. At the substrate concentrations used the hydrolysis of only one ester, nicotinylcholine iodide, was accelerated by the presence of an enzyme. Purified human serum cholinesterase splits nicotinylcholine iodide at roughly the same rate as acetylcholine iodide is split. By contrast nicotinylcholine iodide is not split at all by human red cell cholinesterase (Table 3).

TABLE 3. HYDROLYSIS OF NICOTINYLCHOLINE IODIDE BY CHOLINESTERASES (Substrate concentrations in mM given in brackets)

Enzyme preparation	Acid liberated (μmole ml^{-1} min^{-1})	
	Acetylcholine iodide	Nicotinylcholine iodide
Human serum cholinesterase	81×10^{-3} (25)	74×10^{-3} (8)
Human red cell cholinesterase	85×10^{-3} (8)	0 (8)

When the various esters in increasing concentrations were added to the reaction mixture before acetylcholine they showed no appreciable inhibition of the hydrolysis of the latter compound by red cell cholinesterase.

Effect on neuromuscular transmission

When the compounds were tested in two ways on the neuromuscular transmission (cat sciatic nerve gastrocnemius preparation and rat phrenic nerve diaphragm preparation) only three of the esters were found to have an appreciable neuromuscular blocking effect. The relative molar potencies in comparison with succinylcholine are seen in Table 4. On both test organs the compounds showed the same sequence of activity.

TABLE 4. NEUROMUSCULAR BLOCKING ACTIVITY OF NICOTINYLCHOLINE AND TWO QUATERNARY ANALOGUES

Ester	Cat sciatic nerve gastrocnemius preparation				Rat phrenic nerve diaphragm preparation
	PD 50 (mg/kg)	PD 50 (μM/kg)	Duration	Relative molar potency	Relative molar potency
Succinylcholine iodide	0·004	8×10^{-3}	6 min 20 sec	100	100
Pyridyl-3-acetylcholine iodide methiodide	0·29	6×10^{-1}	5 min 20 sec	1·3	5·1
Nicotinylcholine iodide	0·67	2	1 min 10 sec	0·4	1·7
Nicotinylhomocholine iodide methiodide	3·7	7·5	7 min 20 sec	0·1	<0·1

The neuromuscular blocking effect is considerably less than that for the commonly used neuromuscular blocking agents of naturally occurring or synthetic origin. The duration of recovery from a 50 per cent blockade for two of the compounds was of the same order of magnitude as of that for succinylcholine, when tested after intraarterial injection.

Effect on the respiration

From the experiments shown in Figs. 1–4 it is apparent that all the esters studied had negligible effect on the respiration.

Effect on blood pressure

The effects of the esters on blood pressure at doses of 5–10 mg/kg body weight proved to be extremely variable in the series studied. Thus nicotinylhomocholine iodide methiodide showed no appreciable effect on the blood pressure (Fig. 3). Nicotinylcholine iodide constantly showed a biphasic pressor effect (Fig. 1). Pyridyl-3-acetylcholine iodide methiodide exhibited a fleeting depressor response (Fig. 2) whereas nicotinylcholine iodide methiodide caused a depression of considerable duration (Fig. 4).

Effect on the nictitating membrane

The effect of nicotinylcholine iodide and nicotinylcholine iodide methiodide on ganglionic transmission was measured by the height of contraction of the nictitating

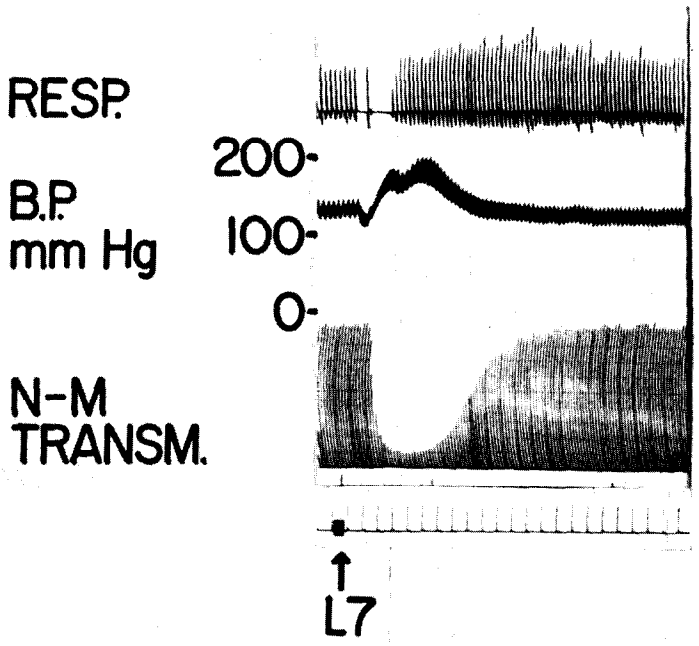


FIG. 1. Effect of nicotinylcholine iodide (L7) on respiration, blood pressure and neuromuscular transmission: 5 mg/kg body weight i.v.; cat 2·1 kg; anaesthetic, allobarbitone; time. 10 sec.

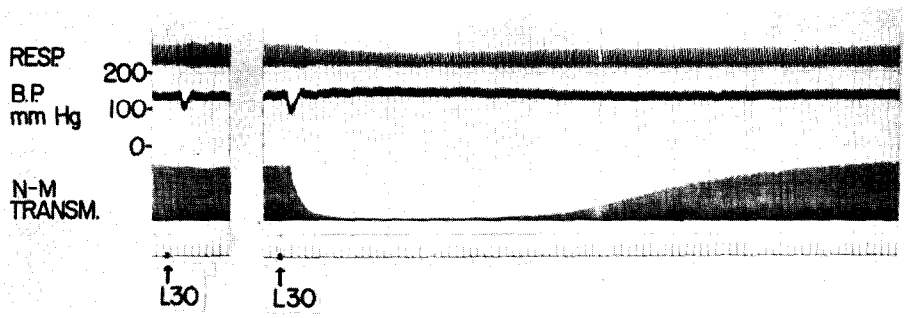


FIG. 2. Effect on pyridyl-3-acetylcholine iodide methiodide (L30) on respiration, blood pressure and neuromuscular transmission: 2·5 resp 5 mg/kg body weight i.v.; cat 2·1 kg; anaesthetic, allobarbitone; time, 10 sec.

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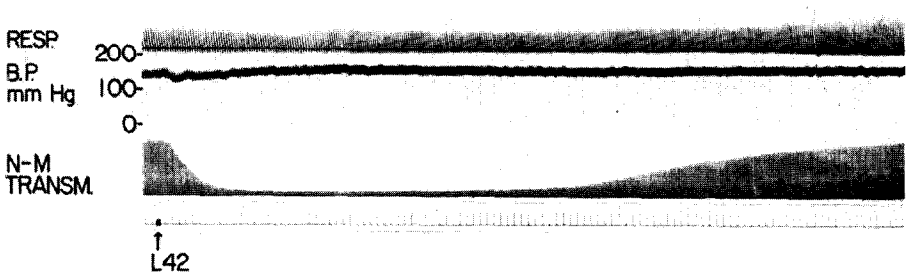

FIG. 3. Effect of nicotinylhomocholine iodide methiodide (L42) on respiration, blood pressure and neuromuscular transmission: 5 mg/kg body weight i.v.; cat 2·1 kg; anaesthetic, allobarbitone; time, 10 sec.

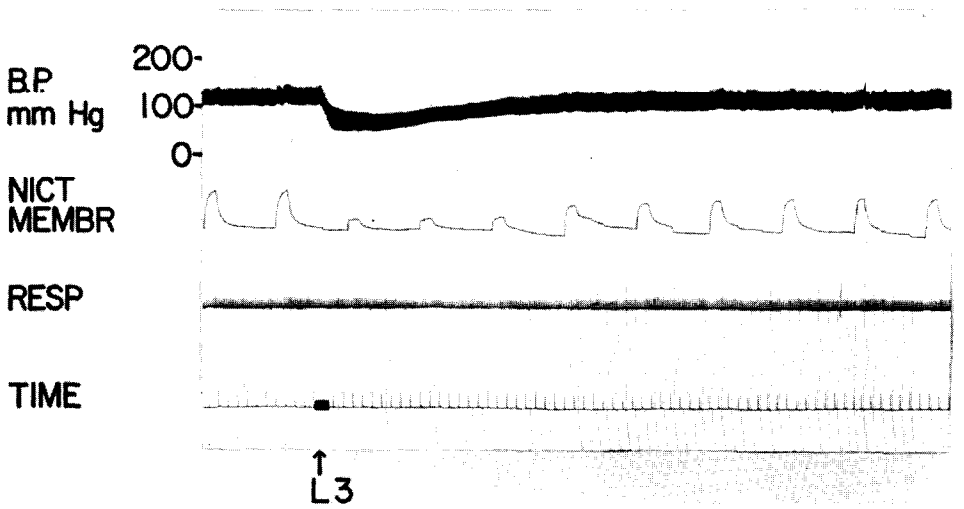

FIG. 4. Effect of nicotinylcholine iodide methiodide (L3) on blood pressure, nictitating membrane and respiration: 20 mg/kg body weight i.v.; cat 2·5 kg; anaesthetic, allobarbitone; time, 10 sec. Intermittent stimulus of isolated central stump of cervical sympathetic trunk 10 sec with rectangular pulses of 0·5 msec duration at a frequency of 20 c/s.

membrane after stimulation of the preganglionic fibres to the superior cervical ganglion at regular time intervals. Nicotinylcholine iodide constantly showed a potentiation of the contractions, whereas nicotinylcholine iodide methiodide decreased the contraction height considerably (Fig. 4). The other esters were inactive on the ganglionic transmission in comparable doses.

DISCUSSION

The synthetic heterocyclic esters in all test organs studied showed much less activity than both murexine[16, 17] and naturally-occurring aliphatic esters.[17, 18] The order of magnitude for a similar response was for the synthetic compounds roughly around 5 mg/kg body weight and for the natural esters 0·1–0·3 mg/kg body weight.

Nicotinylcholine iodide, a compound of considerable theoretical interest as mentioned in the introduction, has the following characteristics: it is easily split by human serum cholinesterase, has a fleeting neuromuscular blocking action, possesses a considerably pressor activity and potentiates the response of the nictitating membrane to preganglionic stimulation. These effects coincide well with the nicotine-like effects of acetylcholine. When nicotinylcholine is converted into the corresponding bisquaternary compound the effect on the blood pressure is transformed into a prolonged depression which coincides with the ganglion blockade (Fig. 4), and is no doubt due to this. Also the effect of nicotinylcholine iodide on the neuromuscular transmission disappears with quaternization. Another feature is that the new compound is no longer hydrolysed by butyrocholinesterase.

The high rate of hydrolysis of nicotinylcholine by butyrocholinesterase is surprising in view of the fact that none of the bisquaternary esters is attacked by butyrocholinesterase but consistent with the well-known high degree of hydrolysis rate of other cyclic choline esters like benzoylcholine previously used as a selective substrate for butyrocholinesterase.[19] The presence of two quaternary groups apparently makes the compounds less attractive for butyrocholinesterase which is believed by some workers[20, 21] to possess no anionic site. The ganglion-blocking activity of nicotinylcholine iodide methiodide could be expected since the distance between the two quaternary nitrogen atoms in this compound is about the same as that which has been found to be optimal for ganglion blocking activity.[2] However, the effect of the nicotinyl derivative is much weaker than that reported for hexamethonium.

The other two bisquaternary compounds although having only one extra atom between the two nitrogens are practically devoid of ganglion blocking action.

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