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THE PHARMACOLOGICAL ACTIONS OF SOME MUREXINE-LIKE SUBSTANCES

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Four choline esters of imidazole acids, two imidazole ethers of choline and thirteen ring-substituted murexine-like compounds were compared with murexine for their muscle-paralysing and their nicotine-like effects. Dihydromurexine appeared, in animal experiments, to be the most potent derivative, but it was shown to be less effective than murexine in man. Among the other compounds, imidazolebutyrylcholine and imidazolepropoxycholine appeared to be worthy of particular consideration. The relation between the chemical structure of the murexine-like substances studied and their pharmacological effects is discussed.

The main pharmacological properties of murexine (urocanoylcholine, [2-β-imidazol-4'(5')-ylacryloyloxyethyl]trimethylammonium chloride hydrochloride), a naturally occurring neuromuscular blocking agent found in the hypobranchial body of some prosobranchiate molluscs, have been described in previous papers by ourselves (Erspamer and Glässer, 1957) and others (Tabachnick and Roth, 1957; Quilliam, 1957; Winbury, 1957; Keyl and Whittaker, 1958).

The investigations described below are concerned with some pharmacological effects of 19 murexine-like derivatives, synthesized by Pasini, Vercellone, and Erspamer (1956) and by Pasini and Coda (1957). Four are murexine analogues (choline esters of imidazole acids), two are imidazole ethers of choline, and 13 are choline esters of acrylic or propionic acids substituted in the β-position with heterocyclic rings different from the imidazole ring.

A few comparative experiments were also carried out with ββ-dimethylacryloylcholine or senecioylcholine, a new choline ester recently discovered by Whittaker (1957) in the marine gastropod *Thais floridana.*

METHODS AND MATERIALS

Murexine Samples.—Synthetic murexine chloride hydrochloride was used throughout the experiments for comparison. Murexine samples were always synthesized shortly before use because the activity of crystalline murexine chloride hydrochloride has been observed to decrease slowly on standing, apparently by as much as 50%. This puzzling fact, not yet fully elucidated by the studies of Pasini and Coda (1957), of course impairs the accuracy of the comparison of murexine with the other substances. The absolute and relative values reported in this paper should, for this reason, be regarded as only approximate. As it was not known whether the derivatives, like murexine, lost activity on standing, they were all freshly synthesized before use.

Pharmacological Methods.—The pharmacological methods used in the present investigation were identical with those previously described (Erspamer and Glässer, 1957).

Neuromuscular blocking action was tested in the intact animal and on the cat sciatic nerve-gastrocnemius muscle preparation, prepared in the manner described by Burn (1950). The doses required to cause head-drop in 50% of rabbits and to paralyse 50% of dogs were determined after rapid intravenous injection. In the cat sciatic nerve-gastrocnemius muscle preparation, maximal stimuli (rectangular current pulses of 7 msec. duration) were applied to the sciatic nerve through a unipolar electrode at a rate of 5 or 10/min. and the contractions were recorded semi-isometrically. Cats were anaesthetized with chloralose (70 mg./kg., i.v.) and the injections were given into the femoral vein. Blood pressure was recorded from the carotid artery. Respiration was recorded by means of a tracheal cannula connected to a tambour. Adequate oxygenation of the blood during apnoea was maintained by a respiratory pump.

Rabbits received intravenous injections into the ear marginal vein, mice into the tail vein.

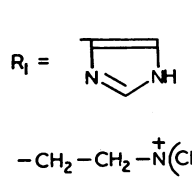
Muscarine-like action was tested on the rabbit intestine and on the rabbit atrium. The isolated frog rectus abdominis muscle and the blood pressure of

TABLE I

COMPARISON OF ACTIVITY OF MUREXINE AND OF SOME IMIDAZOLE ESTERS AND IMIDAZOLE ETHERS OF CHOLINE

Activity of murexine: 100. Dose (mg./kg. body weight of free base) shown in parentheses. The activities of the other drugs are expressed as percentages.

I, Murexine; II, imidazolecarboxylcholine; III, imidazoleacetylcholine; IV, dihydromurexine; V, imidazolebutyrylcholine; VI, imidazolemethoxycholine; VII, imidazolepropoxycholine.



	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)
Mouse: LD50 (i.v.)	100 (6·45)	37 (17·0)	170 (3·8)	115 (5·57)	80 (7·9)	430 (1·5)	290 (2·24)
Rabbit: ED50 (head drop)	100 (0·50)	1·7 (29·0)	25 (2·0)	450 (0·11)	165 (0·3)	26 (1·95)	58 (0·85)
Dog: ED50 (paralysing dose)	100 (0·27)	3·5 (7·4)	67 (0·4)	1,300 (0·022)	—	—	—
Cat: sciatic nerve-gastrocnemius preparation (90 to 100% twitch depression)	100	3	62	330	130	25	95
Spinal cat: blood pressure	100	43	25	420	37	—	<20
Frog: rectus abdominis	100	0·8	40	700	35	0·25	1·7

the spinal cat prepared as described by Burn (1950) were used to test for nicotinic effects.

Murexine-like Compounds.—The following salts of murexine-like compounds were used in our experiments: dihydromurexine dipicrate, imidazolecarboxylcholine chloride hydrochloride, imidazoleacetylcholine dipicrate, imidazolebutyrylcholine dipicrate, imidazolemethoxycholine dipicrate, imidazolepropoxycholine dipicrate; β-pyrid-2-ylacryloylcholine picrate, β-pyrid-2-ylpropionylcholine picrate, β-pyrid-4-ylacryloylcholine dipicrate, β-pyrid-4-ylpropionylcholine dipicrate, β-pyrimidin-2-ylacryloylcholine picrate, β-pyrimidin-4-ylacryloylcholine dipicrate, β-pyrimidin-4-ylpropionylcholine dipicrate, β-pyridazin-3-ylacryloylcholine picrate, β-pyridazin-3-propionylcholine picrate, β-thiazol-2-ylacryloylcholine picrate, β-thiazol-2-ylpropionylcholine picrate, β-fur-2-ylacryloylcholine chloride, β-fur-2-ylpropionylcholine picrate.

Weights of the above compounds were always quoted in terms of their free bases.

RESULTS

Imidazole Esters and Imidazole Ethers of Choline

Table I shows the relative potency of the six new compounds compared with that of murexine. Some absolute values, expressed in mg. free base/kg. body weight, are given in parentheses.

In Table II the paralysing doses in the cat gastrocnemius preparation of murexine (I) are compared with those of the other compounds.

Other pharmacological effects of the substances examined are as follows:

Imidazoleacetylcholine.—Like murexine, imidazoleacetylcholine (2-imidazol-4′(5′)-ylacetoxyethyltrimethylammonium) elicited variable blood pressure responses in the intact dog, cat, and rabbit, following rapid intravenous injection. However, a more or less marked hypertension was predominant after moderate and high doses of the

TABLE II

COMPARISON OF PARALYSING ACTION OF MUREXINE AND OTHER MUREXINE-LIKE COMPOUNDS ON CAT SCIATIC NERVE GASTROCNEMIUS MUSCLE PREPARATION

Numerals in bold type indicate minimum dose required to cause 90 to 100% twitch reduction. The roman numerals refer to the structural formulae in Table I. The doses are expressed in terms of the free base.

Dose (mg./kg.)	Maximum Twitch Reduction (Complete Inhibition: 100%)	Time for 50% Recovery (min.)
Murexine (I)		
0·075	31	3
0·15	74	3·45
0·225	96	8·50
0·375	100	15
0·750	100	33
Imidazolecarboxylcholine (II)		
4·0	40	3
8·0	95	7
Imidazoleacetylcholine (III)		
0·042	25	0·30
0·084	60	1
0·17	85	1·30
0·34	90	1·30
1·70	100	5
Dihydromurexine (IV)		
0·018	46	1·40
0·035	71	2·10
0·07	96	3·20
0·105	99	3·22
0·140	100	4
0·35	100	9
Imidazolebutyrylcholine (V)		
0·035	18	0·10
0·105	81	1·10
0·175	98	3
0·70	100	4
Imidazolepropoxycholine (VI)		
0·06	31	1·40
0·12	78	5
0·18	88	10
0·24	97	15

drug (0.4 to 4 mg./kg.). The pressure rise was sometimes preceded by a transient fall, often accompanied by bradycardia. We observed, in accord with Winbury (1957) and Roth, Rubin, Tabachnick, and Govier (1958), that hexamethonium (2 to 3 mg./kg., i.v.) reduced or abolished only the hypertension, whereas atropine (1 mg./kg., i.v.) eliminated the fall of blood pressure and heart rate. On the other hand, eserine salicylate (50 μg./kg., i.v.) enhanced the rise of pressure in the spinal cat, the size of which (Table I and Fig. 1) was approximately one quarter of that produced by murexine. Similarly, eserine enhanced and prolonged the neuromuscular block exerted by imidazoleacetylcholine in the cat gastrocnemius preparation (Fig. 3).

The rabbit atrium was little affected by this choline ester. Doses of 50 μg./ml., and above, caused only a slight increase in the amplitude and a moderate decrease in the frequency of contraction. Imidazoleacetylcholine elicited a weak contraction of the rabbit colon which was partially antagonized by atropine sulphate (5×10^{-8}). The minimum effective dose was approximately 2 μg./ml.

The minimum concentration of imidazoleacetylcholine active on the leech muscle was 10^{-5}. Previous treatment with eserine caused a fourfold increase in sensitivity and, after eserine, the contracture curve did not reach a plateau so readily as before.

Dihydromurexine (Imidazolepropionylcholine).—The action of dihydromurexine (2-β-imidazol-4′(5′)-ylpropionyloxyethyltrimethylammonium) on

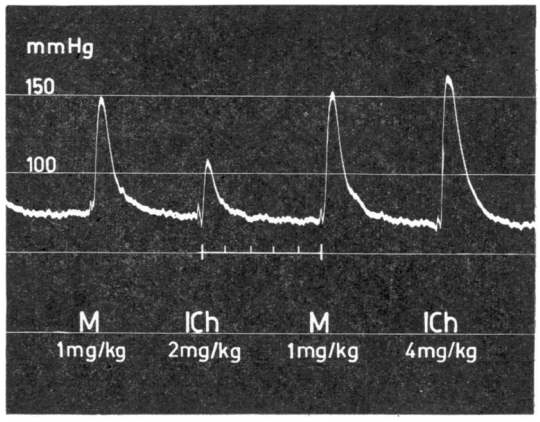


FIG. 1.—Blood pressure of spinal cat. Pressure rise caused by murexine (M) in this experiment was approximately 3·4 times greater than that caused by imidazoleacetylcholine (ICh). Time, 1 min.

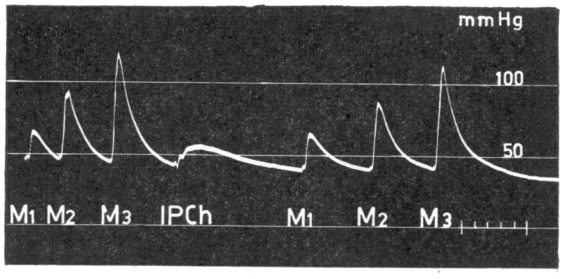


FIG. 2.—Blood pressure of spinal cat. M1, M2, M3 refer to 1, 2, 3 mg./kg. of murexine respectively; IPCh, 5 mg./kg. of imidazolepropoxycholine. Pressure rise caused by 1 mg./kg. murexine was greater, although of shorter duration, than that elicited by 5 mg./kg. imidazolepropoxycholine. Time, 1 min.

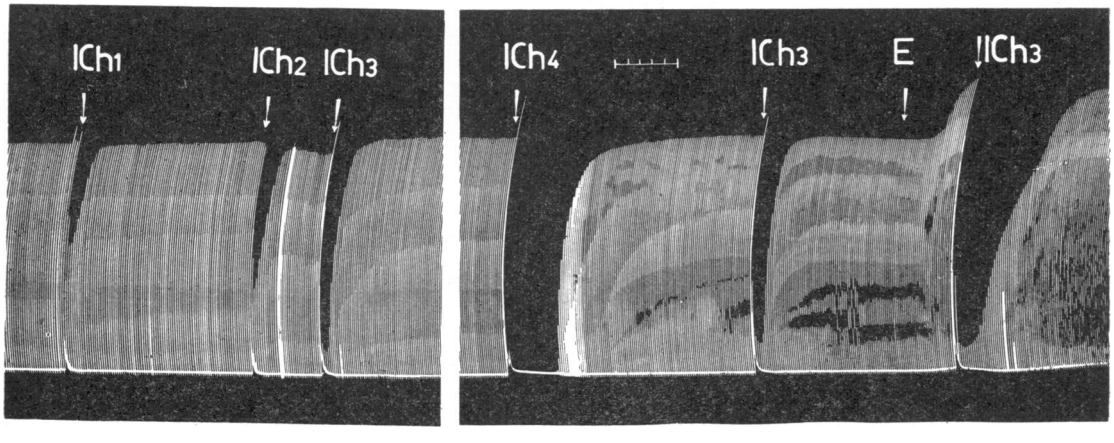


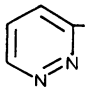
FIG. 3.—Cat sciatic nerve-gastrocnemius muscle preparation. ICh1, ICh2, ICh3, ICh4 represent 0.2, 0.4, 1, 5 mg./kg. of imidazoleacetylcholine respectively; E, 50 μg./kg. of eserine salicylate. All injections were given intravenously. Neuromuscular block, sometimes preceded by increased twitch height, was always short-lasting. Eserine prolonged paralysis markedly. Time, 1 min.

the blood pressure resembled that of murexine and imidazoleacetylcholine. Like Conroy, Kappell, Ferruggia, and Randall (1956), we have found that dihydromurexine was 4 to 5 times more potent than murexine in raising blood pressure in both the cat and the dog. Depending on circumstances, the drug (3 μg./ml., or more) caused either a depression or a stimulation of the rabbit colon. The stimulation was blocked by atropine. Dihydromurexine produced no appreciable effect on the rabbit atrium, in doses up to 10 μg./ml. Other pharmacological effects of dihydromurexine

TABLE III

ACTIVITY OF RING-SUBSTITUTED MUREXINE-LIKE COMPOUNDS COMPARED WITH THAT OF MUREXINE

Activity of murexine: 100. Dose (mg. free bases/kg. body weight) shown in parentheses. The activities of the other derivatives are expressed as percentages.

R_a = $-CH{=}CH-CO-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$ (acrylic series) R_p = $-CH_2-CH_2-CO-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$ (propionic series)		Mouse LD50 (i.v.)	Rabbit Head Drop ED50	Cat Sciatic Nerve Gastrocnemius Muscle	Spinal Cat Blood Pressure	Frog Rectus Abdominis
 Pyrid-2-yl-	R_a	52 (12·7)	110 (0·45)	100	300	800
	R_p	<30 (>20)	<30 (>1·5)	90	—	1,400
 Pyrid-4-yl-	R_a	—	—	<10	15	40
	R_p	—	—	—	—	130
 Pyrimidin-2-yl-	R_a	—	—	<5	<10	<5
 Pyrimidin-4-yl-	R_a	<30 (>20)	—	40	—	115
	R_p	57 (11·3)	—	125	—	750
 Pyridazin-3-yl-	R_a	23 (28·5)	62 (0·8)	20	300	200
	R_p	38 (17·0)	<50 (>1·0)	75	350	900
 Thiazol-2-yl-	R_a	—	<30 (>1·7)	10	—	60
	R_p	—	<35 (>1·5)	10	—	10
 Fur-2-yl-	R_a	—	13 (3·8)	10	—	20
	R_p	—	<25 (>1·8)	10	—	75
ββ-Dimethylacryloylcholine		—	—	60	—	200

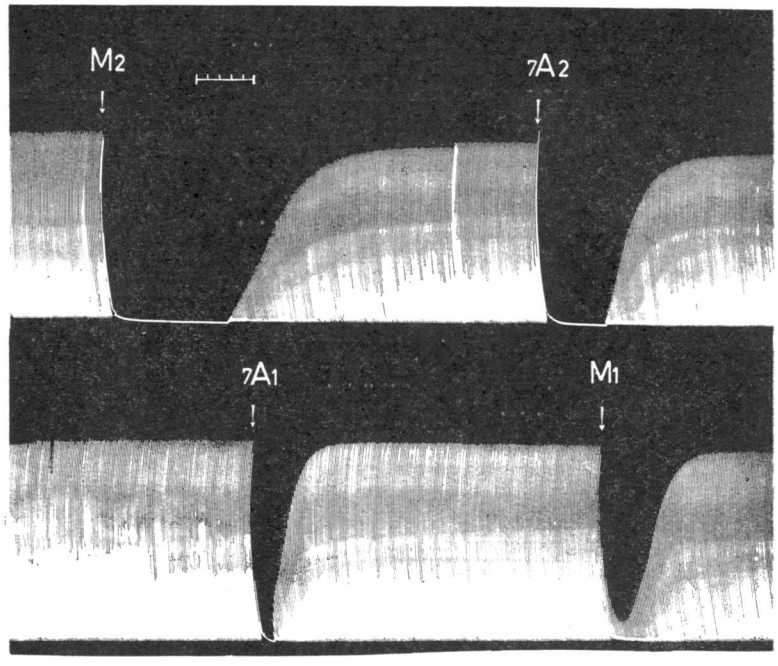


FIG. 4.—Cat sciatic nerve-gastrocnemius muscle preparation. 7A1, 7A2 refer to 0.3 and 1.2 mg./kg. of β-pyridazin-3-ylacryloylcholine respectively; M1, M2 represent 0.2, 0.4 mg./kg. of murexine respectively. Block caused by β-pyridazin-3-ylacryloylcholine was much shorter than that produced by murexine. Time, 1 min.

on the superior cervical ganglion of the cat, on the femoral blood flow of the dog, and on the histamine-induced contraction of the intestinal and tracheal smooth muscle of the guinea-pig have been recently described by Kewitz (1955) and Conroy *et al.* (1956), by Winbury, Wolf, and Tabachnick (1957) and Winbury (1957), and by Tabachnick and Roth (1957), respectively.

Imidazolecarboxylcholine and Imidazolebutyrylcholine.—Both drugs caused a small contraction of the isolated rabbit colon (minimum active dose 2 to 3 μg./ml.). Atropine hardly influenced the action of imidazolecarboxylcholine (2-imidazol-4′(5′)-ylcarbonyloxyethyltrimethylammonium), but effectively blocked that of imidazolebutyrylcholine (2-γ-imidazol-4′(5′)-ylbutyryloxyethyltrimethylammonium). On the rabbit atrium the drugs were ineffective in doses up to 50 μg. and 10 μg./ml., respectively.

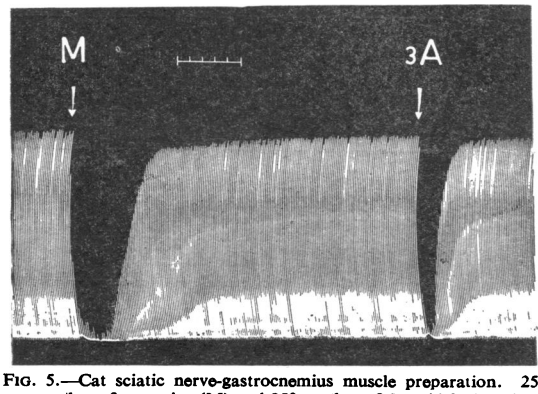


FIG. 5.—Cat sciatic nerve-gastrocnemius muscle preparation. 250 μg./kg. of murexine (M) and 250 μg./kg. of β-pyrid-2-ylacryloylcholine (3A) produced twitch depression of same magnitude. The duration of neuromuscular block was considerably longer for murexine. Time, 1 min.

Imidazolemethoxycholine and Imidazolepropoxycholine.—The minimum dose of both drugs which stimulated the isolated rabbit colon was approximately 2 to 3 μg./ml. Atropine (5×10^{-8}) caused an almost total inhibition of the effect of imidazolepropoxycholine [2-(3-imidazol-4′(5′)-ylpropoxy)ethyltrimethylammonium], but only a 50% inhibition of that of imidazolemethoxycholine (2-imidazol-4′(5′)-ylmethoxyethyltrimethylammonium). In concentrations up to 20 μg./ml. both drugs had little effect on the rabbit atrium. The only action of imidazolepropoxycholine was to cause a slight reduction in the frequency of contraction.

Ring-substituted Murexine-like Compounds

Table III shows the relative potency, on a molar basis, of the ring-substituted compounds, with respect to murexine.

The duration of the neuromuscular block was very short for all substances in this series. Following abolition of the twitch, 50% recovery

was generally observed in 2 to 3 min. (Figs. 4 and 5). Hence, the compounds are rather easily inactivated in the organism.

On the rabbit atrium, both the pyrimidin-4-yl derivatives were ineffective up to concentrations of 20 μg./ml.; β-pyrid-2-ylacryloylcholine showed, at the same dosage, a slight negative chronotropic effect and, occasionally, a slight positive inotropic effect.

Keyl, Michaelson, and Whittaker (1957) examined other ring-substituted choline esters and found that, on the frog rectus preparation, indolylacetylcholine manifested the same stimulant effect as murexine, indolylpropionylcholine 60%, and nicotinylcholine 10% of the effect of murexine. Our investigations confirm their statement that ββ-dimethylacryloylcholine has a very strong nicotinic action, in addition to its remarkable muscle-paralysing effect.

Discussion

The experiments described in this paper were carried out with the main purpose of throwing some light on the problem of the relation between the chemical structure and pharmacological actions of murexine derivatives.

Concerning the first series of the murexine-like compounds examined (the imidazole esters and imidazole ethers of choline) the following conclusions may be drawn from the experimental results:

(*a*) Hydrogenation of the acryloyl side-chain into propionyl caused, as a rule, a powerful increase in both nicotinic and neuromuscular blocking actions. In rabbits, cats, and dogs dihydromurexine was as active as, or even more active than, suxamethonium in producing paralysis of the skeletal musculature. On the frog rectus abdominis dihydromurexine was nearly as potent as acetylcholine in causing contracture.

A remarkable exception is man, for whom the drug is less active than murexine as a muscle-relaxant agent. In fact, in preliminary experiments carried out by De Blasi and Leone (personal communication), dihydromurexine, given by slow intravenous injection in doses up to 1 g., failed to produce consistent muscular relaxation. The reason for this has been explained, at least in part, by the observations of Foldes, Erdös, Baart, and Shanor (1957) and Grelis and Tabachnick (1957), who demonstrated that, whereas murexine is slowly hydrolysed by human plasma cholinesterase, the rate of hydrolysis of dihydromurexine is as fast as that of acetylcholine and 18 times faster than that of murexine.

(*b*) The length of the side-chain of the imidazole acid was of critical importance. A three carbon atom chain seemed to be optimal both for maximal blocking and nicotinic potency. Imidazoleacetyl- and imidazolecarboxyl-choline on the one hand and imidazolebutyrylcholine on the other were less potent than dihydromurexine. Imidazolecarboxylcholine was the least active member of the series, having a paralysing action barely 1% of that of dihydromurexine.

(*c*) Substitution of the ester linkage by an ether linkage might produce different results. In muscle-blocking action determined on the intact rabbit and the cat gastrocnemius preparation, the propoxy derivative appeared to be 1/3 to 1/7 as active as the corresponding propionyl derivative; the methoxy derivative, on the contrary, was 8 to 15 times more active than the corresponding carboxyl derivative. As to its nicotinic effects, imidazolepropoxycholine was approximately 1/20 as effective as dihydromurexine on the blood pressure of the spinal cat (Fig. 2) and 300 to 500 times less effective on the frog rectus abdominis. Both the ethers were more toxic in the mouse than the corresponding esters; it was, however, doubtful whether peripheral respiratory arrest was the only cause of death.

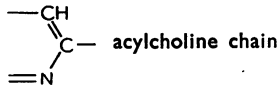
(*d*) Muscarinic actions were very weak, if present, in all compounds of this series. When compared to that of acetylcholine, the spasmogenic action on the rabbit colon was less than 1/10,000 to 1/50,000, the action on the rabbit atrium less than 1%, and the vasodilator action on the dog leg approximately 1% to 2% (Winbury *et al.*, 1957 and 1958; Winbury, 1957).

From Table I it appears that there is no constant correspondence for the different compounds between their paralysing potency as assessed in one animal species and that found in another species; dihydromurexine, for example, is 13 times more active than murexine in the dog, whereas it is only 3 to 5 times more active in the rabbit and on the cat gastrocnemius preparation, and even less active than murexine in man. Differences in the rate of enzymatic hydrolysis by blood and tissue cholinesterases may, as already stated, account for some of these species differences in blocking potency.

Table II shows that the time required for 50% recovery from a given twitch reduction may vary consistently for the different compounds. In this respect, imidazoleacetylcholine (Fig. 3) and imidazolebutyrylcholine produced the shortest neuromuscular block, imidazolepropoxycholine the longest (Fig. 2).

As for the nicotinic effects, the most interesting fact is a frequent dissociation between the nicotinic action on ganglia and adrenal medulla (blood pressure of the spinal cat) and that on the neuromuscular junction (frog rectus abdominis). For example, imidazolecarboxylcholine and imidazolepropoxycholine possess 25% and 20%, respectively, of the activity of murexine on the cat blood pressure, but only 1% to 2% of that on the frog rectus abdominis.

With regard to the second series of our products (the ring-substituted murexine-like compounds), the observations summarized in Table III allow the conclusions: (*a*) that the substitution of a propionyl for the acryloyl side-chain generally causes an enhancement of both the muscle-paralysing and the nicotinic actions; (*b*) that in the more active products the heterocyclic nucleus is linked to the acylcholine chain in such a way as to present the following sequence of atoms (Pasini *et al.*, 1956); and (*c*) that the heterocyclic nucleus



is by no means necessary for the appearance of the characteristic pharmacological effects. ββ-Dimethylacryloylcholine, the choline ester of a simple aliphatic acrylic acid, possesses at least 50% of the muscle-paralysing potency of murexine and a nicotinic effect which is twice as strong.

Another object of this work was that of seeking a product which could be employed, more advantageously than murexine, as an adjuvant in anaesthesia. In fact the advisability of adopting murexine clinically as a muscle relaxant has yet to be established.

The present investigation was virtually unsuccessful in indicating a possible substitute for murexine. Dihydromurexine, which at first seemed on the basis of animal experiments to be the most promising substitute, turned out to be practically ineffective in human beings. Unfortunately, owing to difficulties in their synthesis, imidazolebutyrylcholine and imidazolepropoxycholine could not be prepared in amounts large enough to permit a clinical trial. The substantial attenuation of the disturbing nicotinic effects on blood pressure and on skeletal musculature shown by the second substance could represent an important advantage over murexine.

The ring-substituted murexine-like compounds were not considered for clinical trial. The three or four which seemed worthy of fuller consideration presented very strong nicotinic effects, in addition to an exceedingly short duration of the muscle-paralysing action.

Gruner and Kewitz (1955) have tentatively identified with imidazoleacetylcholine an imidazole derivative found by them in extracts of mammalian brain. It is interesting to note that in our experiments, as in those of Roth *et al.* (1958), imidazoleacetylcholine proved to be one of the less potent of the imidazole derivatives tested. It should be added that Correale (1958) was unable to detect imidazoleacetic acid or imidazoleacetylcholine in brain extracts of lower vertebrates, but succeeded in demonstrating, by paper chromatography, the occurrence of enormous amounts (apparently up to 600 to 800 μg./g. fresh tissue) of another unknown imidazole derivative. This is similar, in many respects, to imidazoleacetic acid and imidazolepropionic acid and, therefore, could perhaps be identical with the imidazole compound obtained by Gruner and Kewitz (1955) following hydrolysis of their new choline ester.

We should like to thank Dr. V. P. Whittaker for a generous sample of ββ-dimethylacryloylcholine.

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