Brit. J. Pharmacol. (1958), **13**, 308.

PHARMACOLOGICAL PROPERTIES OF ββ-DIMETHYLACRYLOYLCHOLINE AND SOME OTHER β-SUBSTITUTED ACRYLOYLCHOLINES

BY

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(RECEIVED MAY 6, 1958)

The mammalian pharmacology of a new naturally occurring ester of choline, ββ-dimethylacryloylcholine (DMAC), has been studied, mainly in the cat, together with that of two synthetic β-substituted acryloylcholines, crotonoylcholine and pent-2-enoylcholine. Comparisons have been made with the reduced form of DMAC, *iso*valerylcholine, with another naturally occurring β-substituted acryloylcholine, murexine (urocanoylcholine), and with suxamethonium. DMAC has been shown to be a ganglion stimulating and neuromuscular blocking agent generally similar, in potency and properties, to murexine. It is also a powerful respiratory stimulant. The other unsaturated esters behaved similarly but were less potent.

ββ-Dimethylacryloylcholine (DMAC, in I, $R_1 = R_2 = CH_3$) has recently been added to the growing number of pharmacologically active esters of choline known to occur in nature (Whittaker, 1957; Keyl, Michaelson, and Whittaker, 1957). It is present in relatively high concentration in the hypobranchial gland of the marine prosobranch gastropod, *Thais floridana* (Southern oyster drill).

This snail is a member of the family Muricidae, other members of which secrete another β-substituted acryloylcholine, murexine (urocanoylcholine) (I, $R_1 = H$, R_2 = imidazol-4(5)-yl) in their hypobranchial glands (Erspamer and Benati, 1953; Keyl *et al.*, 1957).

The mammalian pharmacology of DMAC has now been studied. Two other new β-substituted acryloylcholines, crotonoylcholine (I, $R_1 = H$, $R_2 = CH_3$) and pent-2-enoylcholine (I, $R_1 = H$, $R_2 = CH_3.CH_2-$), have been included for comparison, as have *iso*valerylcholine (the reduced form of DMAC), murexine and suxamethonium. Of these, only murexine occurs in nature, so far as is known. The new ester has been found to resemble murexine (previously studied *inter alia* by Erspamer and Glässer, 1957, and Keyl and Whittaker, 1958) in being a moderately effective neuromuscular blocking agent of the depolarizing type and in having ganglion-stimulating properties. It is also a powerful respiratory stimulant. With murexine, the last two effects are more in evidence in the dog than in the cat, the species mainly studied in the present work. The other new substituted acryloylcholines behaved similarly but were less effective.

MATERIALS AND METHODS

Compounds Used.—Acetylcholine, DMAC, crotonoylcholine, pent-2-enoylcholine, *iso*valerylcholine and suxamethonium (all as iodides, unless otherwise stated) were kindly placed at our disposal by Dr. L.-E. Tammelin, Research Institute of National Defence, Sundbyberg, 4. Murexine (urocanoylcholine) chloride hydrochloride was kindly provided by Professor V. Erspamer. Other compounds used were: dibenamine hydrochloride (Lilly); dihydroergotamine (Sandoz); hexamethonium bromide (Vegolysen, May and Baker); allobarbitone (Dial); eserine salicylate; atropine sulphate (Swedish pharmacopoeia, XIth ed.).

Recording of Respiration, Blood Pressure, and Muscular Contractions.—The effect of DMAC and the other choline esters was studied in over 20 anaesthetized cats. Anaesthesia was induced with allobarbitone (50 mg./kg. body weight) and was maintained by further 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 3 D electroencephalograph.

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 effect of the drugs on neuromuscular transmission was determined by recording isometric contractions of the gastrocnemius muscles 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 S 4, and shielded silver electrodes. The neuromuscular blocking effect of the drugs is expressed in terms of the PD50 (namely, the dose required to produce 50% 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 3 doses and the PD50 and the corresponding duration estimated by graphical interpolation.

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 5 sec. with rectangular pulses of duration of 10 sec., amplitude of 150 mA., and at a frequency of 50 cycles/sec. before and after administration of DMAC and hexamethonium.

Denervation of the Carotid Sinus.—The sinus nerves were freed bilaterally by careful dissection, stimulated to elicit a depressor response, then cut, and the internal carotid artery dissected free, ligated and cut. The vagi were cut on both sides.

Functional Adrenalectomy.—The adrenals were exposed retroperitoneally through lateral incisions on both sides, their blood supply was clamped off by suitable forceps and the experiment continued without removal of the adrenals.

Blood Flow Measurements.—Blood flow was recorded by the method of Lindgren (1958). All injections were given in saline intra-arterially in a volume of 0.1 ml. Acetylcholine was used as a standard.

Frog Rectus Abdominis Muscle Preparations.—The procedure was essentially that of Chang and Gaddum (1933). In this and the following preparation the activity of the esters was expressed as the relative molar potency in percentage units, namely, as the number of moles of acetylcholine giving a response equivalent to 100 moles of active substance at the dose levels compared.

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.

Hydrolysis of Esters by Cholinesterases.—This was measured manometrically at 37° and *p*H 7.4 in 0.023M-$NaHCO_3$ in equilibrium with 5% v/v CO_2 in N_2 in the gas phase (Ammon, 1933) or electrometrically at 25° and *p*H 8 in barbitone buffer as described by Tammelin (1953). The following preparations were used: purified bovine red cell cholinesterase (Winthrop-Stearns Inc.); freeze-dried electric organ from *Torpedo ocellata*; purified human plasma cholinesterase stated to correspond to Cohn's plasma fraction IV-6-3 (AB Kabi). The rate of hydrolysis was expressed in terms of the Q value, namely, μl. acid evolved/mg. enzyme preparation/hr. for acetylcholine or as % of the acetylcholine rate for the other esters. The substrate concentrations, unless otherwise stated, were: acetylcholine, 30 mM (human plasma cholinesterase) or 10 mM (other cholinesterases); other esters, 10 mM. Enzyme concentrations were adjusted to give convenient rates of hydrolysis with the different substrates.

RESULTS

Animal Experiments

Effect on Respiration.—All three unsaturated choline esters stimulated respiration; DMAC was the most powerful respiratory stimulant of the three and evoked a marked response at a dose of 0.1 mg./kg. (Figs. 1 to 6). The effect was noticed immediately upon injection of the compound and did not seem to bear any direct relationship to the neuromuscular blockade. When a biphasic effect on blood pressure was evident, the stimulation of respiration coincided with the first, rising phase (Fig. 2*a*). The animals developed a forced respiration and an increased respiratory rate which lasted less than 1 min. The effect then subsided and in some cases was succeeded by an apnoea due to the hyperventilation.

The respiratory effect of DMAC was moderately potentiated by eserine, somewhat reduced by atropine (Fig. 1), and considerably reduced by the ganglionic blocking agent hexamethonium (Fig. 5). The anti-adrenaline compound dibenamine did not greatly influence the primary respiratory response to the drug, but induced a secondary response which was probably a consequence of the altered effect of the drug on the blood pressure. This can be seen in Fig. 4, where it will be observed that the prolonged fall of blood pressure caused

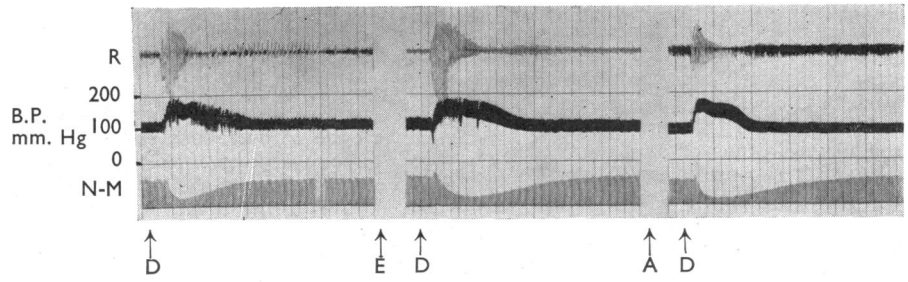

FIG. 1.—Effect of DMAC on respiration (R), blood pressure (B.P.) and neuromuscular transmission (N-M). Cat, 2·1 kg. Anaesthetic, allobarbitone. Time, 10 sec. D = Injection of DMAC (0·25 mg./kg.). E = Injection of eserine salicylate (0·15 mg./kg.). A = Injection of atropine sulphate (2·5 mg./kg.).

by DMAC and *iso*valerylcholine after administration of dibenamine was associated with a more long-lasting hyperpnoea distinct from the primary stimulant effect exerted by DMAC alone.

To find out if the stimulation of the respiration was produced centrally or by way of the chemoreceptors of the carotid sinus, DMAC and *iso*-valerylcholine were compared before and after complete denervation of the sinus and bilateral vagotomy. Denervation of the sinus completely abolished the respiratory stimulant effect of DMAC without significantly affecting the blood pressure and the neuromuscular transmission (Fig. 2). It is therefore concluded that the effect of DMAC is brought about by stimulation of the chemoreceptors of the carotid sinus. The effect of eserine and atropine on the response to DMAC is consistent with what is known about the pharmacology of the carotid sinus (Heymans, 1955).

Clamping off the adrenals had no measurable influence on the respiratory stimulation brought about by DMAC (Fig. 3*b*), showing that the initial respiratory stimulation is not due to a release of pressor amines from the adrenals.

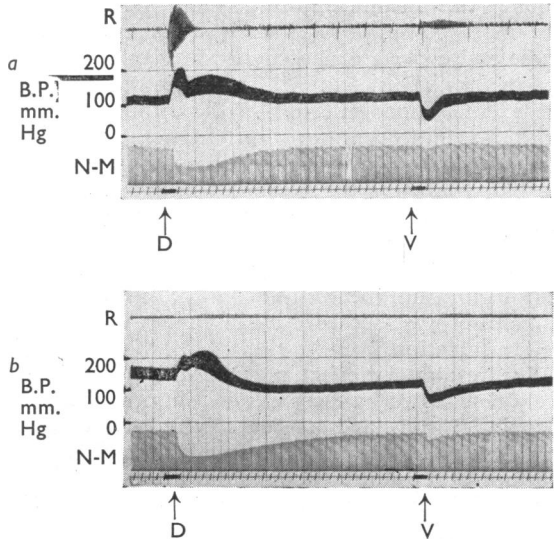

FIG. 2.—Effect of DMAC and *iso*valerylcholine on respiration, blood pressure and neuromuscular transmission. Cat, 2·5 kg. Anaesthetic, allobarbitone. Time, 10 sec. *a* before, *b* after denervation of sinus and cutting of vagi. D = Injection of DMAC (0·1 mg./kg.). V = Injection of *iso*valerylcholine (0·1 mg./kg.).

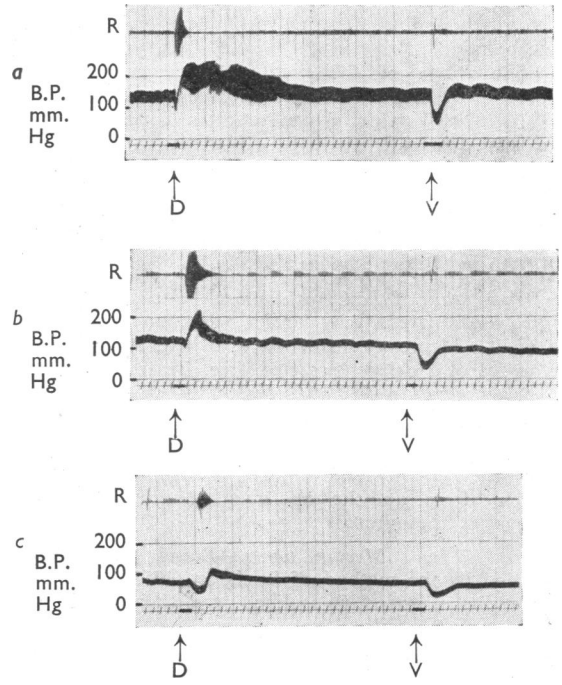

FIG. 3.—Effect of DMAC and *iso*valerylcholine on respiration and blood pressure. Cat, 3·4 kg. Anaesthetic, allobarbitone Time, 10 sec. *a* before, *b* after clamping off blood supply to adrenals, *c* after injection of 1 mg. dihydroergotamine. D = Injection of DMAC (0·1 mg./kg.). V = Injection of *iso*valerylcholine (0·1 mg./kg.).

Effect on Blood Pressure and Nictitating Membrane.—DMAC produces a rise in blood pressure when given intravenously to cats (Figs. 1 to 5). By contrast, pent-2-enoylcholine and crotonoylcholine (in doses of 0.1 to 0.2 mg./kg.) produce a rise in blood pressure only after eserinization and this pressor effect is further potentiated by atropine (Fig. 6), while *iso*valerylcholine has only a depressor effect (Figs. 2 to 4).

When DMAC was injected intravenously, a biphasic effect was sometimes noted (Figs. 2*a*, 3*a*) consisting of an initial rapid rise in blood pressure and then a more sustained pressor effect which gradually wore off. Sinus denervation affected neither of these phases, although abolishing the respiratory stimulant effect completely. When the adrenals were clamped off, only the rapid increase in blood pressure remained (Fig. 3*b*). Dibenamine completely reversed both pressor effects (Fig. 4), as did dihydroergotamine (Fig. 3*c*). Ganglionic blockade by hexamethonium abolished the pressor effect and caused no reversal (Fig. 5). Eserinization or atropinization had very little, if any, effect upon the blood pressure.

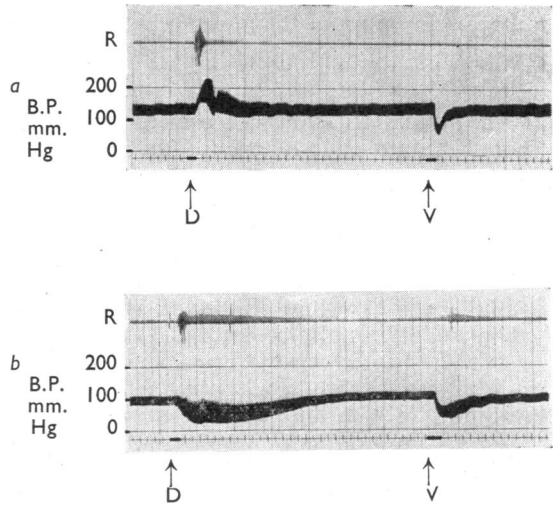

FIG. 4.—Effect of DMAC and *iso*valerylcholine on respiration and blood pressure. Cat, 3 kg. Anaesthetic, allobarbitone. Time, 10 sec. *a* before, *b* 45 min. after the injection of dibenamine hydrochloride (15 mg./kg.). D = Injection of DMAC (0·1 mg./kg.). V = Injection of *iso*valerylcholine (0·1 mg./kg.).

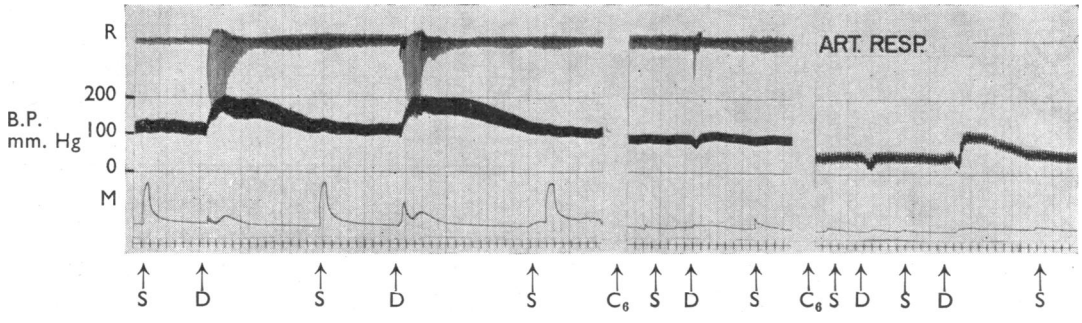

FIG. 5.—Effect of DMAC on respiration, blood pressure and nictitating membrane (M). Cat, 4·5 kg. Anaesthetic, allobarbitone. Time, 10 sec. D = Injection of DMAC; the first injection, 0·1 mg./kg.; the last injection, 1 mg./kg.; the other injections, 0·2 mg./kg. C_6 = Injection of hexamethonium bromide (5 mg./kg.). S = Stimulus of isolated central stump of cat cervical sympathetic trunk for 5 sec. with rectangular pulses of 10 msec. duration at a frequency of 50 c./sec. and intensity of 150 mA.

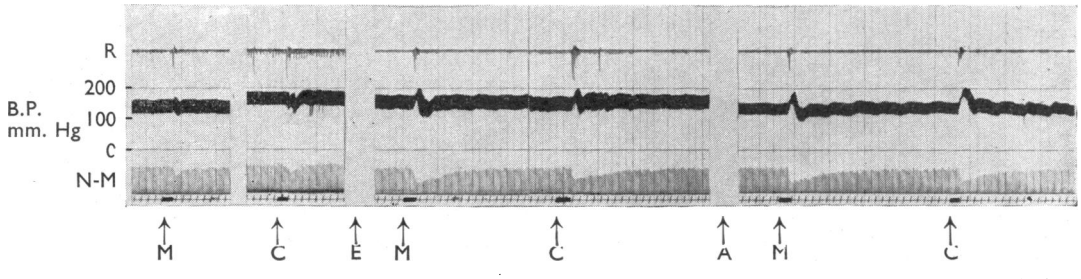

FIG. 6.—Effect of crotonoylcholine and pent-2-enoylcholine on respiration, blood pressure and neuromuscular transmission. Cat, 4·7 kg. Anaesthetic, allobarbitone. Time, 10 sec. M = Injection of pent-2-enoylcholine (0·2 mg./kg.). C = Injection of crotonoylcholine (0·1 mg./kg.). E = Injection of eserine salicylate (0·1 mg./kg.). A = Injection of atropine sulphate (1 mg./kg.).

From these results it is concluded that the first component of the pressor response is caused by sympathetic ganglionic discharge and the second by released pressor amines. That DMAC stimulates the sympathetic ganglia is evinced by Fig. 5, where the effect on the tone of the nictitating membrane of stimulation of the cervical sympathetic and injections of DMAC have been recorded. It will be seen that increasing doses produced increases in tone of the membrane, and both this effect and the effect of stimulation are abolished by the ganglionic blocking agent, hexamethonium. It will also be noted that the stimulation of the superior cervical ganglion by DMAC coincides with the first rapid phase of the pressor effect and also with the period of stimulation of the respiration. It is known that ganglionic stimulation and stimulation of the carotid sinus often run parallel (Heymans, 1955).

Effect on Neuromuscular Transmission.—All three of the new β-substituted acryloylcholines showed neuromuscular blocking activity (Figs. 1, 2, 6, and 7). It will be seen from Fig. 7 that DMAC has about 70% of the potency of murexine

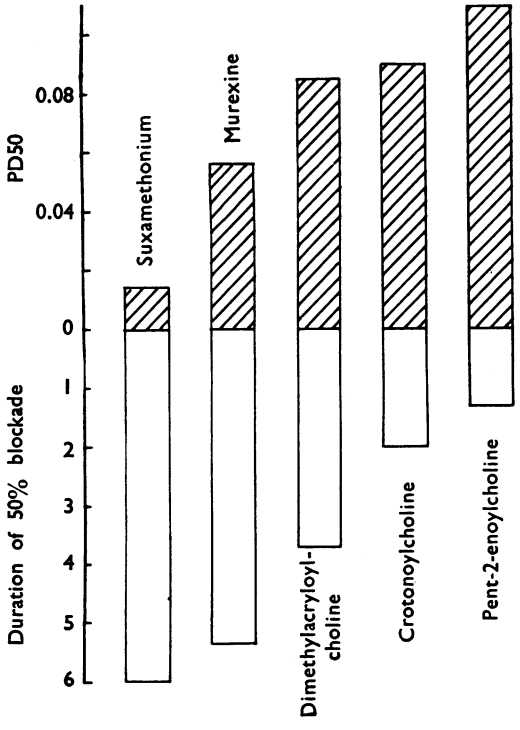

FIG. 7.—PD50 (mg./kg., intra-arterially) and duration of 50% blockade (min.) produced by a series of β-substituted acryloylcholines in the cat. Suxamethonium is included for comparison.

on the basis of either the PD50 or the duration of 50% blockade; crotonoylcholine and pent-2-enoylcholine are less effective than DMAC, in that order. All the β-substituted acryloylcholines are less effective than suxamethonium. The blocking effect of the new compounds was intensified by eserine (Figs. 1 and 6), suggesting that the block was primarily of the depolarizing type.

Activity of Esters on Frog Rectus and Guinea-pig Ileum.—The activities of the three acryloylcholines and *iso*valerylcholine on these preparations are given in Table I. The relative molar potencies of the esters on the frog rectus showed considerable variations in different preparations; the values given in Table I are representative of the present series of results, but are, with one exception, higher than those recorded earlier (Keyl *et al.*, 1957). It will be noted that all the esters are quite active, but none more so than acetylcholine.

TABLE I

ACTIVITY OF β-SUBSTITUTED ACRYLOYLCHOLINES ON FROG RECTUS ABDOMINIS MUSCLE AND GUINEA-PIG ILEUM

Ester	Relative Molar Potency (Acetylcholine = 100)	
	Frog Rectus	Guinea-pig Ileum
DMAC	70	0·13
Crotonoylcholine	16	0·14
Pent-2-enoylcholine	45	1·3
*iso*Valerylcholine	57	0·02

By contrast, the esters showed only very feeble activity on the guinea-pig ileum. Not only were the doses which were needed to elicit a contraction very high, but the response of the muscle differed from that obtained with acetylcholine in that the initial phase of rapid contraction was succeeded by a phase of fluctuating and gradually decreasing tone and maximum contractions could not be elicited. This suggests that the esters were acting by way of the ganglia rather than on the muscle directly.

Effect on Peripheral Blood Flow.—When compared with acetylcholine, which has a well-known peripheral vasodilator effect (Folkow and Uvnäs, 1948; Folkow, Frost, Haeger, and Uvnäs, 1949), the unsaturated esters show a very weak and non-specific activity. DMAC is 1/100th to 1/10th less active than acetylcholine (Fig. 8), but, unlike the latter, increasing doses do not produce increased vasodilatation; indeed, the response if anything decreases (see Fig. 8, injections 7 and 8). Pent-2-enoylcholine seems to be totally without action on

the muscular blood flow in the dose range tested (see Fig. 9, injections 6 to 9), while crotonoylcholine produced a very slight response (see Fig. 9, injections 10 to 13).

In conclusion, the unsaturated choline esters when injected intra-arterially have a very weak vasodilator effect on the muscular blood vessels; this effect is presumably peripheral, since no concomitant changes in blood pressure were observed (Figs. 8 and 9). This is consistent with their feeble effect on the guinea-pig ileum, and in conjunction with the frog rectus and blood pressure results indicates that DMAC and the other unsaturated esters have ganglion stimulating (" nicotine-like ") but no smooth muscle stimulating (" muscarine-like ") activity.

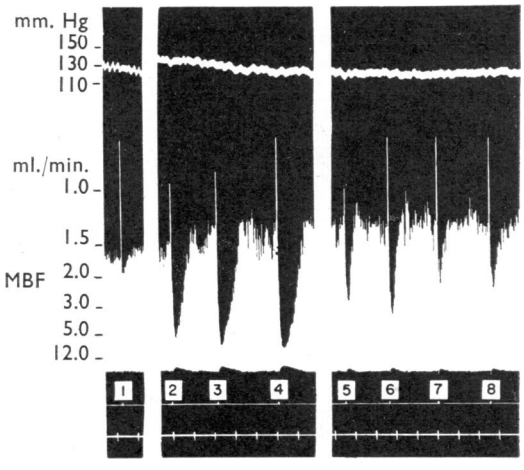

FIG. 8.—Effect of acetylcholine and DMAC bromide on blood pressure and muscular blood flow (MBF) in the right hind limb recorded in the femoral artery. Cat, 2·8 kg. Anaesthetic, allobarbitone. Time, min. The following injections were made intra-arterially in 0·1 ml. saline. 1, saline alone; 2 to 4, acetylcholine (2, 0·001 μg.; 3, 0·1 μg.; 4, 0·1 μg.); 5 to 8, DMAC bromide (5, 6, 0·1 μg.; 7, 8, 1 μg.).

TABLE II

HYDROLYSIS OF β-SUBSTITUTED ACRYLOYLCHOLINES BY CHOLINESTERASES

(a) = determined manometrically, (b) = determined electrometrically, (c) = the same result was obtained electrometrically with 30 mM DMAC and (d) this estimate was based on the assumption that the preparation contained 3% enzyme preparation in stabilizer as stated by the manufacturer.

Preparation	Acetylcholine (Q)	DMAC	Crotonoyl choline	Pent-2-enoyl-choline
		(as % Acetylcholine Rate)		
Human plasm (a) ..	2,700	2 (c)	16	12
Electric organ (b) ..	115	0	—	—
Bovine erythrocyte (a)	$9{\cdot}3 \times 10^4$ (d)	0	1	1

Hydrolysis by Cholinesterases.—The rate of hydrolysis of the new esters by representative cholinesterases is given in Table II. The results show that DMAC is hydrolysed only very slowly by plasma cholinesterase and negligibly by red-cell and electric organ cholinesterases. The other two unsaturated esters were hydrolysed slowly by plasma cholinesterase and just detectably by red-cell cholinesterase. The low rate of hydrolysis of crotonoylcholine by human plasma cholinesterase compared to its optimum substrate, butyrylcholine, shows that the introduction of a double bond into the acyl group greatly retards hydrolysis.

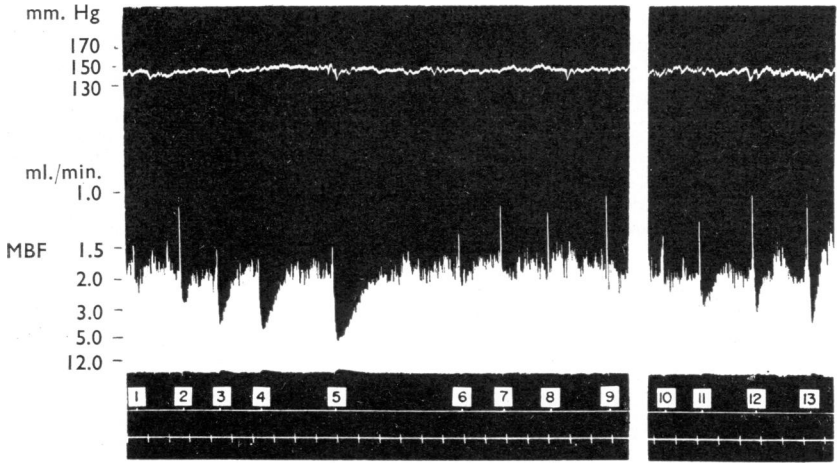

FIG. 9.—Effect of pent-2-enoylcholine and crotonoylcholine, compared with acetylcholine, on blood pressure and muscular blood flow (MBF) in the right hind limb recorded in the femoral artery. Cat, 2·2 kg. Anaesthetic, allobarbitone. Time, min. The following injections were made intra-arterially in 0·1 ml. saline. 1, saline alone; 2 to 5, acetylcholine (2, 0·001 μg.; 3, 0·01 μg.; 4, 0·1 μg.; 5, 1 μg.); 6 to 9, pent-2-enoylcholine (6, 0·1 μg.; 7, 1 μg.; 8, 10 μg.; 9, 100 μg.); 10 to 13, crotonoylcholine (10, 0·1 μg.; 11, 1 μg.; 12, 10 μg.; 13, 100 μg.).

DISCUSSION

DMAC and the two other unsaturated choline esters appear to be the first unsaturated aliphatic choline esters to be investigated pharmacologically, though γ-crotonic betaine esters (Burgen and Hobbiger, 1949), hydroxyalkenyltrimethylammonium compounds (Jacob,

Marszak, Bardisa, Marszak-Fleury, and Epsztein, 1952) and diethylaminoethyl esters of acrylic and senecioic acids (Gilman, Heckert, and McCracken, 1928) have received attention. The new esters are ganglion stimulating and neuromuscular blocking agents; unlike the methyl ester of γ-crotonic betaine they show little or no parasympathomimetic or smooth muscle stimulating action. The new esters are also respiratory stimulants. This action was particularly marked in the case of DMAC and was localized by denervation experiments to the carotid sinus region. Their relative potencies as respiratory and ganglion stimulating agents run parallel; whether this is fortuitous or not is not known, since it is uncertain whether the respiratory stimulation exerted by ganglion stimulating agents is due to a direct action on the chemoreceptors or to a reduction in the carotid blood flow brought about by sympathetic stimulation as suggested by Daly (1954).

The neuromuscular blocking action of the new esters is probably of the depolarizing type, as evinced by the potentiation of the blockade by eserine. Further, Thesleff (personal communication) has found that DMAC and murexine produce a short-lasting membrane depolarization when applied close to a sensitive motor end-plate spot on the isolated tenuissimus muscle of the cat by the ionophoretic micro-application technique (Castillo and Katz, 1957). The depolarization was more prolonged than that produced by acetylcholine. DMAC, like murexine, is only very slowly attacked by cholinesterases, so that it is unlikely that its short-lasting action or the potentiation of its effect by eserine can be ascribed to interactions with cholinesterases. This is in accord with the conclusions reached by Hansson (1958) and Jacob *et al.* (1952) for suxamethonium and other compounds.

Our investigations, thus far, have been concerned only with the mammalian pharmacology of DMAC and throw no light on the rôle of the ester in the hypobranchial gland of *Thais floridana*. The close resemblance between the mammalian pharmacology of murexine and DMAC may, however, extend to their invertebrate pharmacology, and suggests that the rôle of the two esters in their respective species is identical.

One of us (V. P. W.) wishes to express his warm appreciation of the kindness of Professor B. Uvnäs in allowing him to work in the Department of Pharmacology of Karolinska Institutet, and his grateful thanks to the Swedish Medical Research Council, and to AB Vitrum for financial support.

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