SHORT COMMUNICATION

ACTION OF ACETYLCHOLINE ON THE ATROPINISED HEART OF THE FROG DURING SUMMER MONTHS

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Summary : During summer months high doses of acetylcholine produced positive inotropic and chronotropic actions in 19 out of 22 preparations of atropinised perfused heart of the frog. Hexamethonium failed to block both the positive inotropic and chronotropic actions of Ach. Interestingly another ganglion blocking agent mecamylamine as well as beta adrenergic blocking agent propranolol blocked the positive inotropic action but not the positive chronotropic action.

Key words : acetylcholine, atropine, nicotinic action, hexamethonium, mecamylamine, propranolol, frog heart

INTRODUCTION

Action of acetylcholine (Ach) on the atropinised frog heart in winter months has been described previously (5). High doses of acetylcholine fail to produce positive inotropic and positive chronotropic actions on the atropinised heart of the frog in winter (5). High doses of acetylcholine produce positive inotropic and positive chronotropic actions on the atropinised mammalian heart (3,4,9). Acetylcholine induced stimulation of the atropinised mammalian heart is blocked by hexamethonium (3,4,9). We have observed that hexamethonium fails to block the stimulant action of acetylcholine on the atropinised heart of the frog during summer months (July to September). Thus, the action of acetylcholine on the atropinised heart of the frog during summer months has been studied *vis a vis* other antagonists and is the subject of present report.

MATERIAL AND METHODS

The heart of the common Indian frog *Rana tigrina* was perfused through the inferior vena cava by the method of Bulbring as described by Burn (1). The composition of amphibian Ringer, used for perfusing the heart, in *g/litre* was : NaCl 6.5, KCl 0.3, $CaCl_2$ 0.16, $NaHCO_3$ 0.35 and glucose 0.7. 10^{-6} *g/ml* of atropine sulphate was added to the perfusion medium.

The actions of Ach were studied on the atropinised heart of the frog in the absence and presence of hexamethonium (10^{-5} *g/ml*), mecamylamine (10^{-5} *g/ml*) or propranolol (10^{-7} *g/ml*). These antagonists were added to the perfusion medium 1/2 hr before repeating the bolus injection of Ach into the cannula. Ink writing assembly was attached to the writing lever and contractions were recorded directly in ink on the plain paper affixed to the revolving drum. The data was analysed by two tailed Student's t test.

RESULTS AND DISCUSSION

Doses of acetylcholine in the range of 0.1 to 0.8 *mg* were injected, in geometrical progression, into the cannula of the frog's heart being perfused with atropinised amphibian Ringer. Dose of 0.4 *mg* was selected for the studies being reported because it was uniformly effective on different preparations and produced reproducible effect when second such dose was injected on the same preparation at least after an interval of 15 min. Too many doses of acetylcholine specially if administered at relatively shorter interval resulted in tachyphylaxis. Thus, it was not possible to construct the dose-response curve.

Acetylcholine produced an increase in the heart rate as well as an increase in the amplitude of contraction (Fig. 1–A and D) on the atropinized heart of the frog. Hexamethonium did not modify these effects of Ach (Fig. 1-B and Table I). Propranolol blocked the Ach induced increase in amplitude of contraction but did not block the increase in the heart rate (Fig. 1-C and Table I). Mecamylamine also blocked the Ach induced increase in the amplitude of contraction without affecting the heart rate (Fig. 1-E and Table I). Thus, unlike hexamethonium and like propranolol, mecamylamine blocked the stimulant action of acetylcholine on the amplitude of contraction of the atropinised heart of the frog. It is an interesting observation that ganglion blocking agent mecamylamine and beta adrenergic blocking agent propranolol blocked the acetylcholine induced increase in the amplitude of contraction and both these agents failed to block

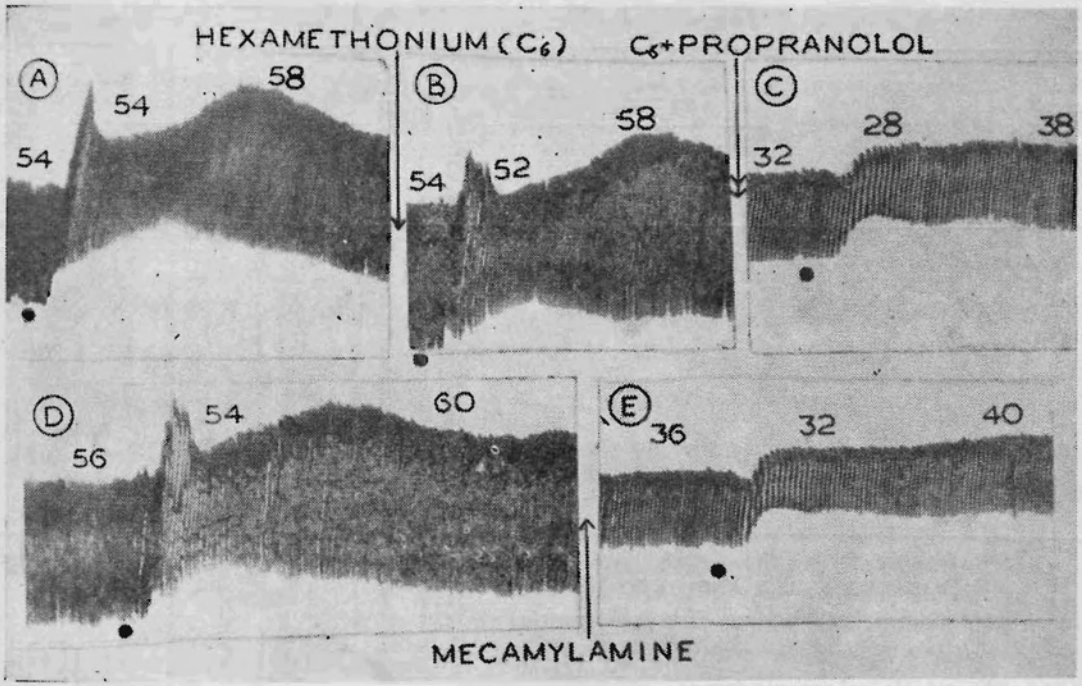

Fig. 1 : *Bolus injection of 0.4 *mg* acetylcholine into the cannula.

Pannels A & D show control nicotinic action produced by acetylcholine on the atropinised heart of the frog. Pannel B shows that hexamethonium has failed to modify the nicotinic action of acetylcholine. Pannels C & E show that propranolol and mecamylamine respectively have blocked the acetylcholine induced increase in the amplitude of contraction on the atropinised heart of the frog. Numura's above indicate heart beats/min.

the positive chronotropic action of acetylcholine on the atropinised heart of the frog. Apparently it seems to be paradoxical that another ganglion blocking agent hexamethonium failed to block both positive inotropic and positive chronotropic actions of acetylcholine on the atropinised frog heart. However, unlike propranolol, mecamylamine failed to block the action of exogenous adrenaline (4 μg injected into the cannula). It may be mentioned that unlike *Rana temporaria* (7), the adrenoceptors of common Indian frog *Rana tigrina*, both in summer and winter months, are beta in nature (6). The beta nature of cardiac adrenoceptors of *Rana tigrina* has been confirmed again during the present study.

TABLE I : Modification of the action of acetylcholine on atropinised heart of the frog by variousblocking agents.

Dose of acetylcholine No. of experiments)	*Blocking agent (concentration)*	*Amplitude expressed as % of control ± S.E.*			*Heart rate expressed as % of control ± S.E.*		
		Before the blocking agent	*After the blocking agent*		*Before the blocking agent*	*After the blocking agent*	
0.4 *mg* (13)	Hexamethonium (10^{-5} *g/ml*)	163.4 ± 18.7	170.9 ± 18.7	P>0.7	119.0 ± 4.6	133.1 ± 8.9	P>0.1
0.4 *mg* (6)	Propranolol (10^{-7} *g/ml*)	161.8 ± 17.1	108.1 ± 8.9	P<0.05	132.1 ± 11.9	108.0 ± 2.7	P>0.1
0.4 *mg* (9)	mecamylamine (10^{-5} *g/ml*)	151.7 ± 9.9	105.3 ± 5.3	P<0.01	113.6 ± 2.2	114.7 ± 3.3	P>0.7

Hexamethonium is known to block the nicotinic action of acetylcholine on the perfused mammalian heart. The adrenergic neurotransmitter in the heart of the frog is adrenaline (2) whereas that of mammalian heart is noradrenaline (2,8). In mammals adrenal medulla releases adrenaline in response to either nerve impulse or acetylcholine. Quaternary ammonium ganglion blocking agents which are markedly effective in blocking the autonomic ganglion can only poorly block the release of adrenaline from the adrenal medulla (8). Hexamethonium is a quarternary ammonium compound whereas mecamylamine is a secondary amine. Naturally, hexamethonium distributes itself mainly in the extracellular fluid whereas mecamylamine is distributed both in extracellular fluid and intracellular fluid. The possibility cannot be ignored that mecamylamine may be blocking the acetylcholine induced increase in the amplitude of contraction of the atropinised frog's heart by acting beyond the common site of action (membrane receptors) of hexamethonium and mecamylamine *i.e.* intracellularly at some step between the excitation of membrane of adrenaline containing chromaffin cells or adrenergic nerve endings of the heart by acetylcholine and release of adrenaline. Why mecamylamine and propranolol block only the positive inotropic action and not the positive chronotropic action of acetylcholine on the perfused atropinised heart of the frog needs to be further explored. In view of the fact that catecholamine stored in mammalian adrenal medulla and the neurotransmitter in the frog's heart is adrenaline, it is worth exploring whether mammalian adrenal medulla also shows differential response to hexamethonium and mecamylamine.

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