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Role of vagus in the antagonism of ouabain induced arrhythmias in dogs by β-adrenoceptor antagonists and related drugs

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The effects of propranolol and related drugs were investigated on ouabain-induced ventricular tachycardia (VT) in dogs with intact and ablated vagi. Propranolol and UM-272 completely antagonized the ouabain VT in dogs with intact vagi, whereas timolol was ineffective. Bilateral vagotomy completely abolished the effect of UM-272 and reduced the effect of propranolol. Diphenylhydantoin, however, reversed ouabain VT in dogs with both intact and ablated vagi. It is inferred that the vagus plays a significant role in the arrhythmolytic effect of propranolol and UM-272.

The role of the autonomic nervous system in the mediation of digitalis toxicity in experimental animals is well documented (Gillis et al 1976). The efficacy of propranolol in the antagonism of ouabain-induced tachy-arrhythmias is also established, the mechanisms underlying this action, however, still remain unclear. This arrhythmolytic effect cannot be explained on the basis of β-adrenoceptor blockade alone, since the concentration needed to achieve this exceeds that required to block the β-receptor (Apantaku et al 1975). Moreover, UM-272, a dimethyl quaternary analogue of propranolol which does not possess β-blocking property, was found to be effective in different models of arrhythmias including that induced by ouabain (Schuster et al 1973). The presence of vagi has been reported to confer protection against the lethal effects of digitalis (Kelliher & Roberts 1976; Gillis et al 1976; Levitt et al 1971). However, the involvement of vagus in the arrhythmolytic action of different drugs in ouabain arrhythmias are not known. In view of the above, we investigated the role of the vagus in the anti-arrhythmic effect of propranolol, UM-272 and some related drugs in ouabain arrhythmias in dogs.

Materials and methods

Mongrel dogs (8–12 kg) of either sex were anaesthetized using pentobarbitone sodium (35 mg kg^{-1}, i.v.). The animals were put on artificial respiration and blood pressure and electrocardiogram (ecg Lead II) were recorded. Arrhythmias were induced by administration of ouabain intravenously in a dose of 40 μg kg^{-1} initially, 20 μg kg^{-1} 30 min later, and thereafter 10 μg kg^{-1} every 15 min until a persistant ventricular tachycardia (VT) developed. Bilateral vagotomy was performed in a group of dogs and ouabain administration in these animals was started 1 h after vagotomy. Ten min after establishment of Ouabain VT, the drugs were administered cumulatively at the rate of 0·5 mg kg^{-1} min^{-1}, i.v. for propranolol, UM-272 and timolol and 5 mg kg^{-1} min^{-1} for diphenylhydantoin. Drug administration was continued till there was reversion of ouabain VT to sinus rhythm or appearance of cardiotoxicity of these drugs.

The drugs used were: (±)-Propranolol HCl (ICI), UM-272 (dimethyl quaternary propranolol) iodide (G. D. Searle & Co), timolol maleate (Merck, Sharp and Dohme), diphenylhydantoin sodium (Epsolin ampoules, Cadila Laboratories) and ouabain (E. Merck). The results were analysed by Student's *t*-test except the incidence of VT and death, where the Chi-square test with Yate's correction was employed for statistical analysis.

Results

Ouabain produced persistant VT at mean doses of 69·5 ± 2·41 μg kg^{-1} (n = 28) and 68·7 ± 2·01 μg kg^{-1} (n = 23) in dogs with intact and ablated vagi respectively. The difference between these two doses was not statistically significant (Table 1). At the time of ouabain VT, there was no significant change in the mean blood pressure in dogs of both intact and ablated vagi groups (Table 2). In the control animals with intact vagi, ouabain VT persisted for at least 90–120 min and spontaneous sinus rhythm reverted in 5 out of 6 dogs within 180 min. On the other hand, of the control dogs of the group with ablated vagi, 5 out of 6 animals died within 30–45 min after development of ouabain VT ($P < 0·025$, compared with the group with intact vagi) and sinus rhythm reverted in only one dog (Table 1).

In the animals with intact vagi, propranolol (3·6–6·8 mg kg^{-1}) UM-272 (2·6–5·0 mg kg^{-1}) and diphenylhydantoin (22–35 mg kg^{-1}) all produced 100% conversion of ouabain VT into sinus rhythm (Table 1), whereas timolol exhibited only partial protection in one of six dogs, at 3·5 mg kg^{-1}. In another 5 dogs, timolol (12, 15, 20, 5 and 8 mg kg^{-1} respectively) failed to afford any protection and the dogs died of cardiac arrest (Table 1). At the peak of anti-arrhythmic activity, propranolol and UM-272 reduced the heart rate whereas the UM-272 also reduced the blood pressure significantly (Table 2). However, the arrhythmolytic doses of diphenylhydantoin failed to alter these parameters to any significant extent.

On the other hand, in bilaterally vagotomized dogs, only diphenylhydantoin showed protection in all four animals studied. UM-272 (4·0–7·2 mg kg^{-1}) failed to afford protection in any of the animals of this group and

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Table 1. Effect of β-adrenoceptor antagonists and related drugs on ouabain induced ventricular tachycardia (VT) in dogs.

Drugs	Treatment Dose in mg kg^{-1} (mean ± s.e.)		Dose of ouabain in μg kg^{-1} (mean ± s.e.)		Incidence* of VT and death Intact vagi		Ablated vagi	
	Intact vagi	Ablated vagi	Intact vagi	Ablated vagi	VT	Death	VT	Death
Control	—	—	68·3 ± 3·07	65·0 ± 2·23	6/6	0/6	6/6	5/6^{b_2}
Propranolol	5·07 ± 0·46	5·75 ± 1·74	68·3 ± 3·07	66·7 ± 3·33	0/6^{a_3}	0/6	5/7^{b_1}	5/7^{b_1}
UM-272	3·76 ± 0·40	5·80 ± 0·48	73·3 ± 4·21	70·0 ± 3·65	0/6^{a_3}	0/6	6/6^{b_3}	6/6^{b_3}
Timolol	10·60 ± 2·56	—	70·0 ± 3·65	—	5/6	5/6^{a_2}	—	—
Diphenylhydantoin	27·50 ± 2·72	29·00 ± 2·41	65·0 ± 9·40	70·0 ± 4·08	0/4^{a_2}	0/4	0/4^{a_2}	0/4

* Values in the numerator indicate the incidence in number of animals and that in the denominator indicate number of animals studied.
^{b_1} $P < 0·05$; ^{a_2}, ^{b_2} $P < 0·025$; ^{a_3}, ^{b_3} $P < 0·01$ (Chi-square test).
^{a_2}, ^{a_3} Data compared with respective control group.
^{b_1}, ^{b_2}, ^{b_3} Data compared with respective intact vagi group.

all the dogs died of ventricular fibrillation (VF) (Table 1). Propranolol reversed ouabain VT into sinus rhythm in only 2 out of 7 dogs; in the other 5 dogs, despite propranolol in doses up to 7·5 mg kg^{-1}, the animals died of cardiac arrest following ventricular fibrillation. Timolol was not studied in the vagotomized group since it was ineffective in the dogs with intact vagi.

Discussion

Ouabain has been reported to induce discharge in both sympathetic and parasympathetic system and the latter effect is said to nullify the deleterious effects of the former in the myocardium (Gillis et al 1976). In the present study the ablation of vagi though not altering the dose of ouabain needed to produce VT, caused greater mortality after ouabain administration, thus corresponding with the reported beneficial effects of vagi on digitalis toxicity (Gillis et al 1976; Kelliher & Roberts 1976).

Propranolol afforded protection in dogs against ouabain VT at doses (Table 1) which were higher than those needed for β-receptor blockade (Apantaku et al 1975). Timolol, a potent β-antagonist, however, failed to afford protection against ouabain VT in most dogs studied, thus agreeing with the results of Mouille et al (1976). Timolol not only failed to reverse the arrhythmia but also exacerbated the condition by increasing fatality probably because of the toxic effects of the drug itself at these doses. The fact that propranolol, which possesses membrane stabilizing property (MSA) and not timolol (which does not) (Shanks 1976) antagonized ouabain VT, stresses the importance of the membrane stabilizing action (Benfey & Varma 1966) in the arrhythmolytic effect of these drugs.

Interestingly, UM-272 (devoid of β-blockade) (Schuster et al 1973) antagonized ouabain VT only in dogs with intact vagi (Table 1). This observation together with the reduction of the arrhythmolytic effect

Table 2. Effect of β-adrenoceptor antagonists and related drugs on the changes in the heart rate and blood pressure produced by ouabain in dogs with intact or ablated vagi.

Treatment**	n	Heart rate min^{-1} (mean ± s.e.)			Mean blood pressure (mm of Hg) (mean ± s.e.)		
			After ouabain			After oubain	
		Before ouabain	Before treatment	After treatment	Before ouabain	Before treatment	After treatment
Intact vagi							
None	6	138 ± 6·8	132 ± 1·9*	128 ± 2·8*	117 ± 6·5	107 ± 4·8	102 ± 4·7
Propranolol	6	142 ± 9·7	150 ± 7·3*	76 ± 4·8^c	113 ± 7·0	104 ± 4·9	93 ± 3·4
UM-272	6	149 ± 13·7	147 ± 12·2*	91 ± 7·7^a	115 ± 8·0	105 ± 5·9	68 ± 4·9^b
Timolol	6	156 ± 10·2	150 ± 13·2*	106†	127 ± 17·7	120 ± 25·3	121†
Diphenylhydantoin	4	155 ± 17·4	158 ± 19·4*	139 ± 17·9	109 ± 10·6	110 ± 7·0	108 ± 7·4
Ablated vagi							
None	6	146 ± 11·7	145 ± 7·9*	136*†	114 ± 8·0	102 ± 7·5	98††
Propranolol	7	158 ± 10·5	169 ± 9·5*	110 ± 22·0††	122 ± 7·1	109 ± 8·5	117 ± 18·0††
UM-272	6	158 ± 14·9	177 ± 19·6*	—	127 ± 3·5	95 ± 8·3	—
Diphenylhydantoin	4	141 ± 9·8	147 ± 12·4*	123 ± 8·6	113 ± 10·5	102 ± 10·2	102 ± 8·6

* Indicates ventricular tachycardia; † one dog survived; †† two dogs survived.
** Doses of drugs used were same as in Table 1.
^{a} $P < 0·025$; ^{b} $P < 0·01$; ^{c} $P < 0·001$ (compared with values before treatment).

of propranolol in dogs with ablated vagi suggests that there is a common denominator, unrelated to β-blockade in the action of these drugs. The abolition of anti-arrhythmic efficacy of UM-272 and reduction of propranolol effect in bilaterally vagotomized dogs cannot be attributed to a general resistance of such arrhythmias to drug treatment since diphenylhydantoin successfully antagonized ouabain VT in animals with intact and ablated vagi (Table 1). The present results clearly indicate the involvement of vagus in the anti-arrhythmic effect of propranolol and UM-272. The fact that both propranolol and UM-272, but not timolol, inhibited cholinesterase enzyme (Alkondon et al 1983) and also exhibited anti-arrhythmic effect suggests a possible correlation between these two effects and supports the above contention. Though there is evidence that diphenylhydantoin may antagonize ouabain arrhythmias by an inhibitory action on cardiac sympathetic neurons (Gillis et al 1971), it can be stated from our experiments that the vagus nerve does not play a significant role in the anti-arrhythmic action of this drug.

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The effects of phenylpropanolamine and other sympathomimetics on food consumption and motor activity in mice

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The effects of phenylpropanolamine on motor activity and on food intake were compared with those of *S*-amphetamine, ephedrine, 2-aminoindane and fenfluramine in groups of mice. Motor activity was additionally measured in mice pretreated with levodopa and benserazide, and food intake in mice pretreated with α-methyl-*p*-tyrosine. Amphetamine (2·5 mg kg^{-1}) increased motor activity, phenylpropanolamine (10–40 mg kg^{-1}) and 2-aminoindane (2·5–10 mg kg^{-1}) decreased activity whilst ephedrine (2·5–40 mg kg^{-1}) had a biphasic effect. Fenfluramine (10–40 mg kg^{-1}) had negligible effect on activity. In mice pretreated with levodopa and benserazide both phenylpropanolamine and 2-aminoindane caused a massive increase in motor activity whilst fenfluramine's action was not affected in the same way. Whilst the anorectic action of fenfluramine was considerably potentiated in mice pretreated with α-methyl-*p*-tyrosine, that of amphetamine, ephedrine, 2-aminoindane and phenylpropanolamine was either unaffected or initially antagonized. It is concluded that the mechanisms of motor and anorectic actions of phenylpropanolamine are similar to those of amphetamine.

Phenylpropanolamine is a widely used sympathomimetic (Editorial 1981, 1982; Pharm. J. 1984). It has been used as an anorectic in the USA and an attempt was made to introduce the drug in the UK for that purpose by mail order (Pharm. J. 1981). Its ready availability contrasts with the severe restrictions imposed on its chemical relative, amphetamine. The mechanism of phenylpropanolamine's anorectic action is unclear (Hoebel 1977).

Drugs chemically related to amphetamine can suppress appetite by different mechanisms. Fenfluramine has been reported to act via tryptaminergic mechanisms whereas amphetamine releases catecholamines (Garattini 1980). We have previously found that these two type substances can be distinguished by pretreatment of mice with α-methyl-*p*-tyrosine (α-mpt). This pretreatment considerably potentiates the anorectic action of fenfluramine, though has little effect, or may even antagonize the anorectic action of amphetamine and similar drugs (Ginawi 1981).

Fenfluramine also differs from amphetamine in that it is sedative. In rodents many amphetamine-like sympathomimetics also decrease locomotor activity but these may be distinguished from fenfluramine by pretreatment with levodopa which unmasks a marked stimulant action.

Using these two pretreatment courses we have characterized the anorectic and motor activity actions of phenylpropanolamine.

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