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EVALUATION OF ANTAGONISM OF ACONITINE-INDUCED DYSRHYTHMIAS IN MICE AS A METHOD OF DETECTING AND ASSESSING ANTIDYSRHYTHMIC ACTIVITY

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1 Antagonism of aconitine-induced dysrhythmias in mice as a method of detecting and assessing antidysrhythmic activity was evaluated.

2 Aconitine-induced dysrhythmias in mice appear to be selectively sensitive to antidysrhythmic agents (administered intraperitoneally) which reduce the inward sodium current in cardiac cells.

3 Antidysrhythmic agents whose mechanism of action is thought to depend on β-adrenoceptor blockade, prolongation of cardiac monophasic action potentials or calcium antagonism are ineffective in delaying the onset of aconitine-induced dysrhythmias in mice. The inactive drugs were practolol, sotalol, bretylium, amiodarone and verapamil.

4 Comparisons of anti-dysrhythmic activities of test drugs should be based on more than one ED value and should take account of efficacy as well as potency.

5 The mouse aconitine test is a useful and rapid method of evaluating oral antidysrhythmic activity in terms of potency, efficacy and duration of action.

6 With respect to potency, efficacy, oral activity, duration of action and safety, 3α-amino-5α-androstan-2β-ol-17-one hydrochloride (Org 6001) offered the most satisfactory overall profile of the active drugs tested (Org 6001, aprindine, quinidine, disopyramide, lignocaine, mexiletine, procainamide and propranolol).

Introduction

Protection against aconitine-induced dysrhythmias in rodents has commonly been used as a method of detecting and assessing anti-dysrhythmic activity (Vargaftig & Coignet, 1969; Szekeres & Papp, 1971; Dadkar & Bhattacharya, 1974; Nwangwu, Holcslaw & Stohs, 1977) Estimation of the efficacy of antidysrhythmic agents is based either on their ability to interrupt an established dysrhythmia or on their ability when administered prophylactically to protect against the development of the dysrhythmia. In the former type of experiment, the ED_{50} value is defined as the dose of test compound required to interrupt the dysrhythmia for at least 1 min in 50% of the treated animals (Szekeres & Papp, 1971). In the latter, the ED_{50} values are variably defined as (a) that dose of test compound required to produce a positive response in 50% of the animals where a positive response is the mean control value + 2 s.e. of the time to the appearance of dysrhythmias (Nwangwu *et al.*, 1977); (b) the dose required to increase the lethal dose of aconitine by 50% (Dadkar & Bhattacharya, 1974) or (c) the dose of test compound required to increase the arrhythmogenic dose of aconitine by 50% (Vargaftig, Sugrue, Buckett & van Riezen, 1969). Since log dose-response lines to anti-dysrhythmic agents are frequently non-parallel and potency and efficacy are not synonymous, valid comparisons of the anti-dysrhythmic activities of test compounds are difficult to make by these methods. The purpose of the present study was, therefore, to compare known anti-dysrhythmic agents in terms of potency, efficacy and toxicity, using a mouse aconitine test and also to study in more detail the selectivity of this test. The drugs used in this study represented all four classifications of antidysrhythmic drugs (Vaughan Williams, 1970).

Method

A modification of the method described by Dadkar & Bhattacharya (1974) was used. Preliminary studies indicated that mice heavier than those commonly used (12 to 25 g) gave more consistent responses to antidysrhythmic agents. Male mice (CE.CFLP) in the

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weight range 30 to 45 g were therefore used. The animals were anaesthetized with sodium pentobarbitone 60 mg/kg given intraperitoneally 15 min prior to aconitine infusion. The electrocardiogram (Standard lead II) was recorded on a Devices M2 Recorder from subcutaneous steel needle electrodes. Aconitine nitrate (10 μg/ml in distilled water) was infused from a Palmer constant output pump at a rate of 1.27 μg/min via a steel needle inserted into a tail vein. The intraperitoneal and oral routes were chosen for drug administration in order to avoid any acute haemodynamic effects resulting from direct drug action consequent upon intravenous administration. Drugs given intraperitoneally were administered 30 min before aconitine infusion. Those given orally were administered (through a blunt ended needle inserted into the oesophagus via the mouth) 1 h before aconitine infusion. Control animals were given vehicle alone via the appropriate route. The sequence of electrocardiographic events during aconitine infusion in mice has been documented by Dadkar & Bhattacharya (1974) and by Nwangwu *et al.* (1977) and consists of the appearance of premature ventricular systoles (PVS), ventricular tachycardia (VT), ventricular fibrillation (VF) and finally cardiac arrest. Routine studies in our laboratories using this model have indicated that although this sequence of events is extremely consistent in control animals, some drug-treated animals show an abrupt transition from PVS to VF. Also in instances where aconitine infusion is markedly prolonged by an anti-dysrhythmic agent, cessation of respiration may precede VF. The time taken to the onset of the first PVS was therefore chosen as the end point and the amount of aconitine (μg/kg body wt.) required to induce this dysrhythmia calculated. Six to ten animals were used for each dose of each drug tested and a separate set of control values obtained for each drug tested. All drugs used were dissolved in distilled water and given in a volume of 0.1 ml/100 g body wt. Drug doses are expressed as mg of salt per kg body wt.

Statistical analysis of the results

Significant differences between drug-treated groups and control groups were first of all determined by applying Student's *t* test to the raw data. The percent increase in the amount of aconitine required to induce dysrhythmias in each drug-treated animal (above the mean value in the appropriate control group) was then calculated and log dose-response lines constructed by linear regression analysis. The slopes of the regression lines to specific pairs of drugs were compared by two-tailed *t* tests and examined for differences at the 5% level. ED_{50} and ED_{100} values were defined as the doses of test drug required to raise the dysrhythmic dose of aconitine by 50% and 100% respectively above that required in control animals. Relative drug potencies were compared by obtaining 95% confidence limits for the ED_{50} and ED_{100} values. Non-intersecting confidence limits were taken to signify a difference in drug potencies.

Drugs used

Drugs used were propafenone hydrochloride (Helopharm), aprindine hydrochloride (Eli Lilly), quinidine sulphate (Merck), disopyramide phosphate (Searle), lignocaine hydrochloride (MacFarlan Smith), mexiletine hydrochloride (Boehringer Ingleheim), procainamide hydrochloride (Squibb), (±)-propranolol hydrochloride (Imperial Chemical Industries), practolol hydrochloride (Imperial Chemical Industries), sotalol hydrochloride (Bristol Laboratories), bretylium tosylate (Burroughs Wellcome & Co.), amiodarone hydrochloride (Labaz), verapamil hydrochloride (Knoll A.G.) and 3α-amino-5α-androstan-2β-ol-17-one hydrochloride (Org 6001) (Organon).

Results

Drugs administered intraperitoneally

Org 6001, aprindine, disopyramide, lignocaine, mexiletine, procainamide, quinidine, propafenone (2'-[2-hydroxy-3-(propylamino)-propoxy]-3-phenylpropiophenone) and propranolol were all effective in antagonizing dysrhythmias induced by aconitine. Figure 1 shows log dose-response lines to each of these compounds and Table 1 lists the calculated ED_{50} and ED_{100} values together with the 95% confidence limits. Using the ED_{50} value as a measure of potency, Org 6001 and propranolol had the lowest ED_{50} values and were significantly ($P < 0.05$) more potent than lignocaine, mexiletine and procainamide. The latter drug had the lowest ED_{50} value and was significantly less active than any of the other compounds. However, when the ED_{100} value was used to compare potency, propranolol emerged as one of the least potent compounds being significantly less potent than Org 6001, aprindine, disopyramide or quinidine. As can be seen both from Figure 1 and Table 1, the log dose-response line to propranolol had the shallowest gradient, the slope of the dose-response line being significantly different from that of dose-response lines to procainamide, lignocaine, quinidine, disopyramide and aprindine. It should be noted that the ED_{100} value for propranolol could only be obtained from extrapolation of the log dose-response line since a dose equivalent to the ED_{100} value was lethal. Hence, propranolol, although potent, is lacking in efficacy. A similar argument can be put forward for mexiletine. The slopes of the log dose-response lines

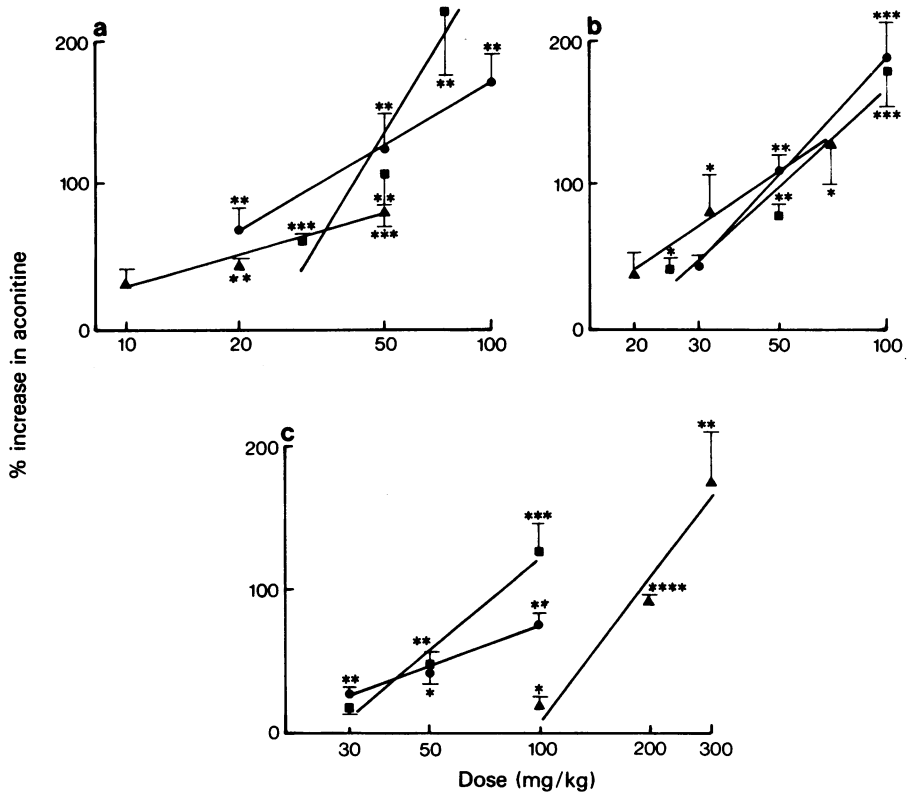

Figure 1 Log dose-response lines to Class I antidysrhythmic agents given intraperitoneally. The ordinates are the % increase in aconitine required to induce premature ventricular systoles compared to controls. In (a), (●) Org 6001; (■) aprindine and (▲) propranolol. In (b), (●) disopyramide, (■) quinidine and (▲) propafenone. In (c), (●) mexiletine, (■) lignocaine and (▲) procainamide.
$*P < 0.05$; $**P < 0.01$; $**P < 0.001$; $****P < 0.0001$ (significantly different from control).

Table 1 ED_{50} and ED_{100} (mg/kg) values calculated from log-dose response lines to Class I antidysrhythmic agents

Drug	ED_{50}	ED_{100}	*Slope*	*Class*
Org 6001	15 (3–25)	30 (17–48)	146	I
Propafenone	24 (0.05–40)	41 (29–9462)	167	I
Aprindine	30 (15–39)	41 (27–51)	389	I
Quinidine	33 (23–39)	57 (43–64)	233	I
Disopyramide	34 (25–38)	50 (41–56)	280	I
Lignocaine	*47 (37–54)	*84 (66–107)	208	I
Mexiletine	*57 (47–74)	*190 (131–542)	91	I
Procainamide	*107 (76–132)	*194 (145–260)	199	I
Propranolol	19 (14–37)	*106 (57–160)	71	I & II

Drugs were given intraperitoneally.
In parentheses, 95% confidence limits are given. ED_{100} values for mexiletine and propranolol could only be obtained by extrapolation since these doses were toxic.
* Denotes a significant difference ($P < 0.05$) from the Org 6001 values.

to propafenone, procainamide, lignocaine, quinidine, disopyramide and Org 6001 were similar and the order of potency was the same regardless of which ED value was used for comparison. However, the slope of the regression line to aprindine was significantly higher than that to propranolol, mexiletine and Org 6001.

With the exception of Org 6001 and quinidine, all drugs so far discussed induce marked side effects (e.g. motor incoordination, paraesthesia, convulsions, A–V block) when given in doses of from three to five times their intraperitoneal ED_{50} values.

In contrast to the above drugs, practolol, sotalol, bretylium (given either 30 min or 3 h before aconitine), amiodarone or verapamil were not effective in delaying the onset of aconitine-induced dysrhythmias (Table 2). These drugs were given in doses of from 20 to a maximum of 200 mg/kg or until toxicity resulted.

Drugs administered orally

All drugs tested (Org 6001, aprindine, quinidine, disopyramide, lignocaine, mexiletine, procainamide and propranolol) were effective in antagonizing dysrhythmias evoked by aconitine. Log dose-response lines to these agents are depicted in Figure 2 and the ED_{50} and ED_{100} values listed in Table 3. Comparing the ED_{50} values, the order of potency was essentially similar to that observed when drugs were given intraperitoneally; Org 6001, propranolol and aprindine had the lowest ED_{50} values, Org 6001 and aprindine being significantly more potent than disopyramide, mexiletine and procainamide. Again, procainamide was significantly less potent than any of the other compounds (with the exception of lignocaine). As in the intraperitoneal studies, the oral potency order changed when considering the ED_{100} values. Aprindine became significantly more potent than any of the other drugs tested (again reflecting the steepness of the regression line). Indeed the slope of the log dose-response line to aprindine was significantly greater than that to any other drug. Of the remaining drugs, Org 6001 had the lowest ED_{100} value and was significantly more potent than lignocaine or procainamide. Propranolol became the sixth least potent. Consistent with the intraperitoneal studies, the slope of the regression line to propranolol was significantly shallower than that to quinidine, disopyramide and aprindine and the ED_{100} value could only be obtained by extrapolation. Lignocaine showed a similar lack of oral efficacy in non-toxic doses. Again, the poor oral activity is reflected in the slope. The ED_{100} value for mexiletine was lower than that for lignocaine but mexiletine could only be given in doses of up to 1.9 times the oral ED_{50} value. This suggests that the efficacy of oral mexiletine is limited by a narrow therapeutic ratio.

Comparison of oral tolerance to antidysrhythmic agents

Table 4 summarizes the most serious toxic effects observed following oral administration of antidysrhythmic agents. Lignocaine and mexiletine were the most toxic of the drugs tested. Convulsions were induced following doses corresponding to 1.1 and 1.9 times their oral ED_{50} values respectively. Aprindine and propranolol induced convulsions at doses equal to five times the oral ED_{50} value. Motor incoordination was observed in animals given disopyramide at a dose equivalent to 3.5 times its ED_{50} value. In contrast, the only obvious toxic effect noted following administration of Org 6001 at ten times it ED_{50} value was ptosis. Convulsions were not seen and motor activity appeared to be normal.

Duration of action of orally active agents

Org 6001, disopyramide, procainamide, propranolol, quinidine and aprindine were examined for duration of action. Groups of six animals were given oral doses of drugs corresponding to twice the oral ED_{50} values and aconitine infused at 1 to 18 h after administration. The results are summarized in Table 5. Significant antidysrhythmic activity was present 6 h after administration of Org 6001, aprindine and disopyramide, 4 h after procainamide, 2 h after quinidine and 1 h after propranolol. Antidysrhythmic activity appeared to be present 18 h after oral dosing with aprindine although the results were not statistically significant.

Table 2 The amounts of aconitine required to induce premature ventricular systoles in untreated mice and in mice given 50 mg/kg (intraperitoneally) of Class II, III or IV antidysrhythmic agents

	Aconitine (μg/kg)		
Drug	*Control*	*Treated*	*Class*
Practolol	55.4 ± 6.2	*30.3 ± 3.1	II
Sotalol	49.6 ± 3.3	45.6 ± 8.0	II & III
†Bretylium	42.5 ± 4.2	38.0 ± 5.3	III
Amiodarone	47.0 ± 5.1	55.3 ± 6.2	III
Verapamil	51.5 ± 3.9	55.2 ± 5.4	IV

Values are mean ± s.e. mean.
The amounts of aconitine required to induce dysrhythmias were not significantly increased by any of the drugs tested.
* The amount of aconitine required to induce dysrhythmias was significantly less ($P < 0.05$) than that required in control animals.
† Bretylium was given in a dose of 20 mg/kg since higher doses induced convulsions and/or death.

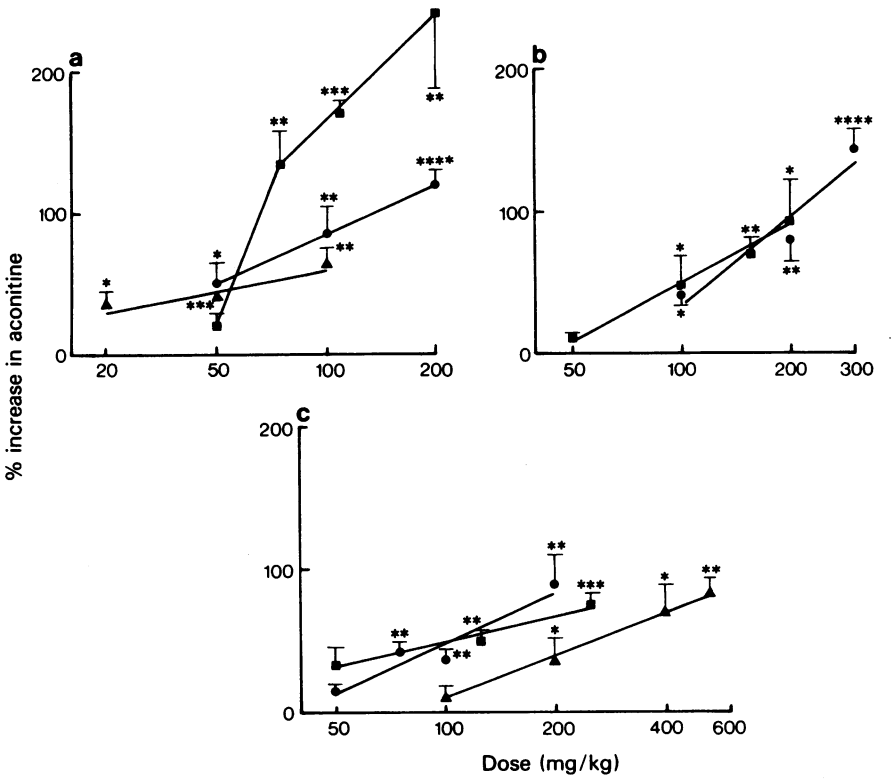

Figure 2 Log dose-response lines to Class I antidysrhythmic agents given orally. The ordinates are the % increase in aconitine required to induce premature ventricular systoles compared to controls. In (a), (●) Org 6001; (■) aprindine; (▲) propranolol. In (b), (●) disopyramide; (■) quinidine. In (c), (●) mexiletine; (■) lignocaine and (▲) procainamide.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ (significantly different from controls).

Table 3 ED_{50} and ED_{100} (mg/kg) values calculated from log-dose response lines to Class I antidysrhythmic agents

Drug	*ED_{50}*	*ED_{100}*	*Slope*	*Class*
Org 6001	52 (15–73)	132 (96–235)	114	I
Aprindine	55 (37–64)	*66 (58–85)	351	I
Quinidine	101 (67–140)	202 (148–570)	158	I
Disopyramide	*115 (84–140)	210 (175–280)	192	I
Lignocaine	112 (39–241)	*841 (318–5.6 × 10^6)	56	I
Mexiletine	*107 (79–139)	285 (185–773)	117	I
Procainamide	*268 (181–386)	*845 (516–3203)	98	I
Propranolol	49(8–116)	449(162–4.6 × 10^{10})	54	I & II

Drugs were given orally.
In parentheses, 95% confidence limits are given. ED_{100} values for lignocaine, mexiletine and propranolol could only be obtained by extrapolation since these doses were toxic. The ED_{100} for procainamide was also obtained by extrapolation but this dose was not tested for toxicity.
* Denotes a significant difference ($P < 0.05$) from the Org 6001 values.

Discussion

The results of the present study indicate that dysrhythmias induced by aconitine in mice are sensitive to antidysrhythmic agents of the local anaesthetic type. Previous workers have shown that Org 6001 (Salako, Vaughan Williams & Wittig, 1976), propafenone (Neuss & Buss, 1978), aprindine (Steinberg & Greenspan, 1976) propranolol (Coltart & Meldrum, 1971) mexiletine (Singh & Vaughan Williams, 1972a), disopyramide and quinidine (Sekiya & Vaughan Williams, 1963), lignocaine (Vaughan Williams, 1970) and procainamide (Szekeres & Papp, 1971) all reduce the maximum rate of phase 0 depolarization of cardiac intracellular action potentials. These antidysrhythmic drugs would therefore be categorized as possessing a Class I action according to Vaughan Williams' (1970) classification. In contrast, antidysrhythmic agents lacking a Class I action were ineffective in delaying the onset of aconitine-induced dysrhythmias. These were the β-adrenoceptor antagonists, practolol and sotalol (Class II); agents which prolong the duration of the cardiac action potential, amiodarone, bretylium and sotalol (Class III) (Singh & Vaughan Williams, 1970; Wit, Steiner & Damato, 1970; Cabasson, Mellet, Gulmond, Bachy, Sassine & Peuch, 1975) and the calcium antagonist verapamil (Class IV) (Singh & Vaughan Williams, 1972b). Thus aconitine-induced dysrhythmias in mice appear to be selectively sensitive to Class I antidysrhythmic agents. This is not surprising since Tanz (1974) has shown that aconitine-induced automaticity and tachycardia in cat papillary muscle is the result of an enhancement of the inward sodium current.

The second point to emerge from this study is that comparison of antidysrhythmic activity based on only one arbitrarily defined ED value is not sufficient to evaluate fully the activity of antidysrhythmic agents,

Table 4 Comparison of oral tolerance of antidysrhythmic agents

Drug	*Dose (mg/kg)*	*Multiple of oral ED_{50} value*	*Toxic effects*
Org 6001	250	5.0	Ptosis (1/6)
Org 6001	500	10.0	Ptosis (4/6)
Aprindine	200	3.6	Motor incoordination
Aprindine	276	5.0	Convulsions
Quinidine	390	4.0	Ptosis (2/6)
Quinidine	780	8.0	Convulsions
Disopyramide	400	3.5	Shivering, motor incoordination
Lignocaine	125	1.1	Convulsions
Mexiletine	200	1.9	Convulsions
*Procainamide	536	2.0	No obvious side effects
Propranolol	244	5.0	Convulsions

* Procainamide was not studied at a higher dose.

Table 5 Duration of action of antidysrhythmic agents administered via the oral route: amounts of aconitine (μg/kg) required to precipitate premature ventricular systoles are shown

		Time (h) of aconitine infusion after treatment				
Drug	Control	1	2	4	6	18
Org 6001	45.0 ± 3.7	*75.0 ± 9.0	***77.0 ± 2.9	***81.1 ± 5.1	**74.4 ± 4.6	42.2 ± 4.5
Disopyramide	50.4 ± 1.8	*184.2 ± 41.8	*118.8 ± 16.9	***118.5 ± 6.9	*111.6 ± 12.1	40.7 ± 5.2
Procainamide	77.6 ± 3.3	*141.1 ± 8.8		*131.8 ± 12.6	88.7 ± 5.6	85.8 ± 3.4
Propranolol	72.0 ± 3.6	*106.5 ± 9.1	80.4 ± 6.7	74.5 ± 3.5	66.1 ± 2.6	
Quinidine	71.0 ± 5.1		**111.0 ± 9.6	90.0 ± 10.1	79.7 ± 7.6	64.7 ± 8.1
Aprindine	56.8 ± 1.9	***152.6 ± 6.3		*122.3 ± 16.5	**77.3 ± 4.3	71.1 ± 12.3

Figures are mean ± s.e. mean.
* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ denote significant differences from the control values. Drugs were given in doses equivalent to twice their oral ED_{50} values.

indeed this can be misleading. The outstanding example in this respect is propranolol which has previously been identified as one of the most potent (Dadkar & Bhattacharya, 1974) or most active (Nwangwu *et al.*, 1977) antidysrhythmic agents in this test. The present results would suggest that although an antidysrhythmic response can be elicited with a low dose of propranolol, the efficacy of this drug in antagonizing aconitine-induced dysrhythmias is very limited. Propranolol, in non toxic doses, conferred a far lesser degree of protection against aconitine-induced dysrhythmias than any of the other Class I agents tested. This was true following either intraperitoneal or oral administration. Of the remaining Class I compounds, Org 6001, propafenone, aprindine, quinidine, disopyramide, lignocaine and procainamide given intraperitoneally offered a marked degree of protection against dysrhythmias; Org 6001 had the lowest ED_{50} and ED_{100} values and was significantly more potent than lignocaine, mexiletine and procainamide. Procainamide was the least potent. Mexiletine was intermediate in efficacy between propranolol and the remaining Class I agents.

The results from the oral studies indicate that lignocaine was essentially inactive following oral administration. Doses of only up to 1.1 times the ED_{50} value could be given. This is in keeping with the known lack of oral activity of lignocaine in man (Prescott, Clements & Pottage, 1977). Mexiletine was orally active. However, in comparison with the other Class I antidysrhythmic agents tested, the oral efficacy of mexiletine was poor. This drug could only be tolerated in doses of up to 1.9 times the oral ED_{50} value whilst the log dose-response line was relatively shallow. In contrast, aprindine, Org 6001, quinidine and disopyramide offered substantial protection against the development of dysrhythmias in non-toxic doses. Of these compounds disopyramide and quinidine were approximately equipotent. The log dose-response line to aprindine was relatively steep. Consequently, this agent emerged as equipotent with Org 6001 when comparing ED_{50} values but substantially more potent when comparing ED_{100} values. However, the relatively shallower log dose-response line to Org 6001 and the lack of toxicity at high doses suggests that good antidysrhythmic activity is obtained over a wide dose range. This may be important clinically where adequate plasma levels have to be maintained for long periods of time. Procainamide was also found to possess oral activity. However, the doses required to elicit an antidysrhythmic response were large and, although doses greater than twice the ED_{50} value were not given, this dose was well tolerated.

Studies on the duration of action of orally active agents given at twice their oral ED_{50} values suggested that aprindine, Org 6001 and disopyramide were the longest acting of the agents tested. Substantial antidysrhythmic activity could be demonstrated 6 h after administration of these drugs and although the result was not statistically significant, aprindine appeared to be active 18 h after dosing. Procainamide was active for 4 h and quinidine for 2 h. Excluding Org 6001 which has not been orally studied in man, these results are in line with those obtained clinically. Aprindine has a half life in man of 100 h (Prescott & Pottage, 1978), disopyramide a half life of up to 22 h (Hulting & Jansson, 1977) and procainamide and quinidine half lives of 3 and 6 hours respectively (Prescott & Pottage, 1978). A study on the duration of action of mexiletine was not carried out because of the toxicity associated with this compound.

The results obtained from the present study also imply that Org 6001 was the best tolerated following oral administration of all the active drugs tested. The only obvious sign of toxicity when given in a dose equivalent to ten times its oral ED_{50} value was ptosis. Quinidine was the next best tolerated. All other orally active agents induced motor incoordination or convulsions in doses of from 3.5 to 5 times their oral ED_{50} values.

Taken together, the results obtained from this study would imply that with respect to potency, efficacy, oral activity, duration of action and safety, Org 6001 offers the most satisfactory overall profile. Aprindine was more potent (when given orally) and probably longer acting than Org 6001 but its safety margin was considerably smaller. Disopyramide was somewhat less potent and again more toxic than Org 6001. Procainamide and quinidine were shorter acting. Lignocaine was essentially orally inactive and propranolol lacking in efficacy.

In conclusion, aconitine-induced dysrhythmias in mice allow a rapid evaluation of antidysrhythmic activity provided that the limitations of the test are taken into account. The model appears to be selective for antidysrhythmic agents whose mode of action involves a reduction in the inward sodium current in cardiac cells. Drug potency should be assessed using more than one ED value and efficacy should be observed. The model is also useful in estimating duration of action of antidysrhythmic agents since results obtained are compatible with those observed in man.

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