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The activity of β-adrenoceptor blocking agents in protecting mice from the cardiotoxic effects of ouabain

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A method is described for the assessment of the protective activity of drugs against the lethal effect of intravenously infused ouabain in mice. Electrocardiographic studies confirmed that protection is exerted against a cardiotoxic action of the glycoside. Activities are quoted for some β-adrenoceptor blocking agents. Both (−)- and (+)-enantiomers of propranolol were active but only the (−)-enantiomer of sotalol and INPEA—drugs devoid of membrane-stabilizing properties.

The anti-arrhythmic activity of β-adrenoceptor blocking agents has been assessed by determining their protective action against experimental arrhythmias induced by chloroform (Sekiya & Vaughan Williams, 1965) or cardiac glycosides such as ouabain (Vaughan Williams & Sekiya, 1963). Lawson (1968) has described a method for determining protective action against chloroform-induced arrhythmia in mice. The method described here similarly provides a simple and rapid means of assessing such protection against ouabain cardiotoxicity in mice.

METHODS

Male CFW mice, 20–25 g, were sedated by the intraperitoneal injection of 10 mg kg^{-1} diazepam and placed in a thermostatically controlled cupboard at an ambient temperature of 30°. Thirty min later they were infused via a tail vein with a solution of ouabain in saline, 1 mg ml^{-1}, at the rate of 0·05 ml every 10 s, until cardiac fibrillation could be detected by palpation of the chest wall; this end-point was rapidly followed by death. The survival time was thus determined for each mouse to the nearest 5 s. Groups of 10 mice readily gave mean survival times, with an estimate of precision. Protection by a test compound was assessed by the prolongation of survival time in groups of mice pretreated by intraperitoneal injection of a dose of the compound 30 min before ouabain infusion. Dose-response relations for protection were obtained from the survival times of mice treated with at least three doses. They were compared by computation of doses required for a standard prolongation of the survival time from the control value of 105·75 ± 1·3 s to 130 s (termed the ED130). This value was obtained, together with its 95% confidence limits, from the regression relation computed by the method of least squares, according to the equation quoted in Diem & Lentner (1970).

In a second set of experiments the mice were inserted in a Perspex holder before ouabain infusion. This holder was perforated in two places such that needle electrodes could be introduced to pierce the skin of the mouse in positions appropriate for recording the ecg. This was done by means of a Tektronix storage oscilloscope, 564B, one trace being stored every 10 s during the intravenous infusion of ouabain,

0·5 mg ml^{-1}, 0·05 ml every 10 s until a characteristic change in the wave form occurred (Fig. 1). This consisted of a prolongation of duration of the QRS complex and elevation of the T wave. The infusion period necessary for this change to develop could thus be determined for each mouse and the protective effect of test compounds expressed by the dose computed to increase this period from the control value of 78·5 ± 3·0 s to 105 s (termed the ED105).

Drugs were dissolved in saline, with the exception of diazepam, which was diluted from the ampoule solution. They included diazepam (Roche); lignocaine (Pharmaceutical Manufacturing Company); ouabain (BDH); (±)-, (+)- and (−)-propranolol & practolol (by courtesy of Dr. B. Newbould, ICI); quinidine (BDH); (±)-, (+)- and (−)-sotalol (by courtesy of Dr. G. McKinney of Mead Johnson); (±)- and (−)-INPEA (*N*-isopropyl-*p*-nitrophenylamine) and α-methyl INPEA (by courtesy of Messrs. Selvi, Milan).

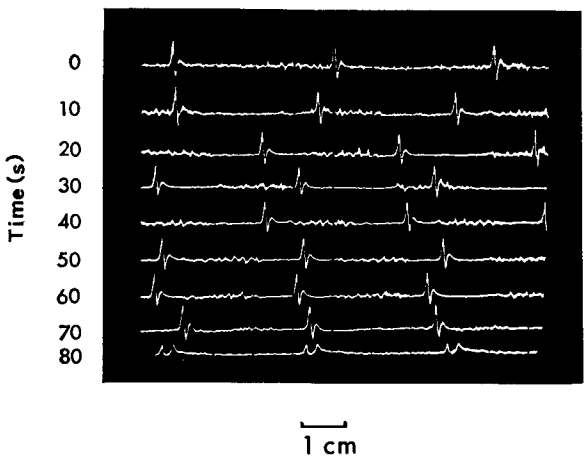

FIG. 1. Oscilloscope sweeps from ecg leads before and at 10 s intervals after commencing infusion of a mouse with ouabain, 0·5 mg ml^{-1}, 0·05 ml every 10 s via a tail vein. Sweep speed 30 cm s^{-1}.

RESULTS

The use of diazepam was necessary to reduce spontaneous movements sufficiently to obtain reliable ecg traces. Preliminary experiments had shown that prior injection of diazepam considerably increased the survival time of mice infused with ouabain but this was shown to be associated with a fall in body temperature ($r = -0{\cdot}75$; $P < 0{\cdot}001$) consequent upon the reduced activity. When mice were kept at an ambient temperature of 30°, diazepam no longer caused a fall in temperature and the survival time to ouabain infusion did not differ significantly from that of mice untreated with diazepam and kept at laboratory temperature. Moreover, regression relations computed for survival time and dose of (±)-propranolol showed no significant difference between control mice and sedated mice at 30° either for coincidence or parallelism (Table 1).

Regression relations were derived from both the survival time to ouabain infusion, and the time for appearance of the change in ecg waveform adopted as endpoint, with various doses of β-adrenoceptor blocking agents and other drugs in mice pre-treated with diazepam and kept at 30° (Fig. 2). Protective activities of the drugs tested

Table 1. *Survival times for mice infused intravenously with ouabain*, 1 mg ml^{-1}, 0·05 ml *every* 10 s *after pretreatment with* (±)-*propranolol, intraperitoneally, with or without additional diazepam*, 10 mg kg^{-1}, *intraperitoneally and maintained at* 30°.

Dose (±)-propranolol (mg kg^{-1})	Mean survival time (s) ± s.e. Sedated	Non-sedated
5	115·0 ± 1·5	112·9 ± 1·5
10	124·4 ± 2·6	125·0 ± 1·3
20	136·9 ± 1·6	131·25 ± 1·2
40	141·0 ± 2·9	138·3 ± 2·1
ED130 (with 95% confidence limits)	14·6 (12·3–17·5)	18·4 (16·1–21·5)

F values for coincidence of relations = 0·55 ($P>0·2$).
for parallelism = 3·73 ($P>0·05$).

against both effects are shown in Table 2 in the form of ED130 and ED105 values, with their 95% confidence limits.

DISCUSSION

The method described constitutes a simple means of determining protective activity against the toxic effects of ouabain in mice, corresponding to that described by Lawson (1968) for activity against chloroform-induced arrhythmia. The results suggest that it can readily yield an indication of potential anti-arrhythmic activity otherwise requiring the study of ouabain-induced arrhythmia in larger animals for its assessment (Vaughan Williams & Sekiya, 1963).

Confirmation that the lethal effect of ouabain studied is a consequence of cardiotoxic action is provided by the values shown in Table 2, which clearly indicate similar

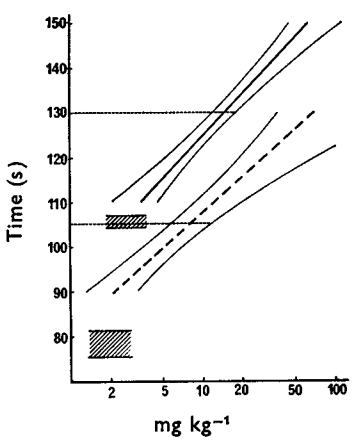

FIG. 2. Regression relations for survival time (continuous line) and time to ecg endpoint (broken line) and dose of (±)-propranolol, in mice infused intravenously with ouabain. The envelopes enclose the 95% confidence limits.

The hatched areas indicate the mean values ±S.E. for mice untreated with propranolol; for survival time, 105·75 ± 1·3 s and for ecg endpoint, 78·5 ± 3·0 s.

For the effect of propranolol on survival time, r = 0·85, b = 31·0 ± 7·4, n = 29. For effect on ecg endpoint, r = 0·72, b = 26·0 ± 8·9, n = 48.

Table 2. *Standard effective doses, with 95% confidence limits, of drugs protecting mice from effects of intravenous ouabain infusion after intraperitoneal injection.*

Drug	Lethal end-point ED130 (mg kg^{-1})	ECG end-point ED105 (mg kg^{-1})
(±)-Propranolol	14·6 (12·3–17·5)	7·9 (5·7–11·6)
(−)-Propranolol	5·7 (2·8–8·3)	4·3 (3·6–5·3)
(+)-Propranolol	19·9 (16·6–24·5)	10·9 (8·1–15·2)
(±)-Sotalol	5·6 (2·2–9·9)	5·35 (3·9–7·7)
(−)-Sotalol	6·4 (5·6–7·3)	3·9 (2·7–5·1)
(+)-Sotalol	Inactive up to 40	Inactive up to 40
(±)-INPEA	Inactive up to toxic doses	Slightly active at 100
(−)-INPEA	66·0 (54·2–92·3)	38·8 (30·5–54·6)
α-Methyl INPEA	217 (181–313)	Confused end point
Practolol (oral)	124·6 (99·6–153·7)	60·2 (48·0–70·1)
Quinidine	24·3 (20·6–30·9)	10·0 (7·0–13·6)
Lignocaine	59·6 (48·5–81·9)	49·0 (33·6–68·6)

relative activities against the two effects of ouabain for the drugs studied. The coefficient of correlation for the two sets of values is 0·96.

The mouse is resistant to the toxic effects of ouabain and the dose found here to be certainly lethal, of about 20 mg kg^{-1}, agrees with that recorded elsewhere (Barnes & Eltherington, 1964); it represents some 60 times the lethal dose in the guinea-pig (Barnes & Eltherington, 1964; Dohadwalla, Freedberg & Vaughan Williams, 1969). It is thus not surprising that the doses of drugs needed to prolong survival by 25% are greater than those affording similar protection in the guinea-pig (Dohadwalla & others, 1969), though the ratio of effective doses for (±)-propranolol in the two species is much less than 60. Even so, the standard effective doses reported here for propranolol and quinidine were both less than the reported ED50 against chloroform-arrhythmia in mice by Lawson (1968), by about threefold.

Dohadwalla & others (1969) and Singh & Vaughan Williams (1970) have discussed the evidence that both β-adrenoceptor blockade and membrane-stabilizing properties can contribute to anti-arrhythmic activity and antagonism to the cardiotoxic effects of ouabain. In confirmation of the findings of Barrett & Cullum (1968) in cats and dogs and of Dohadwalla & others (1969) in guinea-pigs, we find that (+)-propranolol, which has very little β-adrenoceptor blocking activity, shows a protective activity against ouabain toxicity. The activity of this isomer approaches that of the racemic form, though it is only one-third that of the (−)-form, which blocks the β-adrenoceptor; both isomers show local anaesthetic and quinidine-like properties (Barrett & Cullum, 1968).

Lignocaine and quinidine also protected against ouabain toxicity, confirming the effectiveness of membrane-stabilizing properties for this action, unaccompanied by β-adrenoceptor blocking activity. On the other hand, the protection afforded by practolol and the (−)-enantiomer of sotalol (MJ1999)—drugs which lack membrane-stabilizing properties (Singh & Vaughan Williams, 1970)—suggests that β-adrenoceptor blockade alone can be responsible for this effect; the (+)- enantiomer of sotalol, which is weak in this respect (Patil, 1968) proved inactive against ouabain. Similarly, the (−)-enantiomer of INPEA, which is also weak in membrane-stabilizing properties (Singh & Vaughan Williams, 1971), but is responsible for all the β-adrenoceptor

blocking activity of this compound (Patil, 1968; Almirante & Murmann, 1966) showed protective activity against ouabain toxicity, though of a low order, such that activity could not be established for (±)-INPEA below toxic doses. α-Methyl INPEA, which is only very weakly active on cardiac β-adrenoceptors (Somani, 1969), also showed only slight activity in this situation.

Singh & Vaughan Williams (1970) report that sotalol markedly prolongs the duration of the cardiac action potential and consider that this may have contributed to the protection of guinea-pigs against ouabain-induced arrhythmia. It is not known whether this property resides in both optical isomers though, as the (+)-enantiomer did not protect mice against ouabain toxicity it may be devoid of this property also. Both the racemic and (−)- forms of sotalol were, however, more active in this situation, relative to propranolol (Table 2), than reported by other workers from comparisons of the β-adrenoceptor blocking, quinidine-like or local anaesthetic properties of these two drugs, though they show comparable β-adrenoceptor blocking activity by the oral route in man (refs. in Singh & Vaughan Williams, 1970). It is thus possible that the third anti-arrhythmic property of Vaughan Williams may contribute to the protective action of sotalol against ouabain cardiotoxicity in mice.

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