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Investigations of the mode of action of a new antidysrhythmic drug, Kö 1173

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Summary

1. Kö 1173, 1-(2′,6′-dimethyl-phenoxy)-2-amino-propane, was equal to lignocaine in potency as a local anaesthetic on stripped frog sciatic nerve at pH 7·5.

2. In anaesthetized guinea-pigs in which ventricular fibrillation had been produced by an intravenous infusion of ouabain, intravenous administration of Kö 1173 caused reversion to sinus rhythm in ten out of ten animals. The mean dose of Kö 1173 required was 3·3 mg (15·3 μmol)/kg. When the ouabain infusion was continued, ventricular fibrillation did not recur, and the lethal dose of ouabain was greater than in controls.

3. Pretreatment with Kö 1173, however, did not influence the toxicity of ouabain infusion, implying great brevity of action.

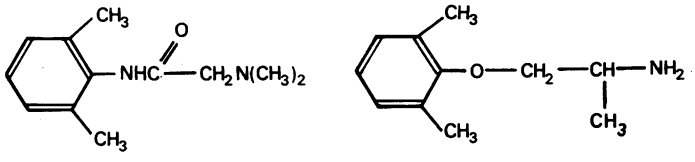
4. Kö 1173 given intravenously caused a bradycardia which was maximal in 2–3 min, and which did not last more than 5 minutes.

5. Kö 1173 reduced the maximum driven frequency of isolated rabbit atria, raised electrical threshold, slowed conduction velocity and depressed contractions.

6. Kö 1173 reduced the maximum rate of depolarization of intracellularly recorded rabbit atrial and ventricular potentials, but did not affect the resting potential or the duration of the action potential.

Introduction

The compound Kö 1173, 1-(2′,6′-dimethyl-phenoxy)-2-amino-propane, which bears some resemblance to lignocaine (Fig. 1), was found in unpublished experiments with animals to have an anticonvulsant potency comparable to that of diphenylhydantoin and phenobarbitone, but had less sedative effect than phenobarbitone. Diphenylhydantoin has been much used in recent years for the control of cardiac dysrhythmias, (evidence reviewed by Dreifus & Watanabe, 1970), and it was, therefore, thought of interest to investigate the effects of Kö 1173 on cardiac muscle.



Lignocaine KÖ 1173

FIG. 1. Structural formulae of Kö 1173 and lignocaine.

The protective action of Kö 1173 against ouabain-induced ventricular fibrillation has been studied in anaesthetized guinea-pigs, and its effects on contractions, conduction velocity, electrical threshold, spontaneous frequency, and maximum driven frequency have been measured in isolated rabbit atria.

There has been some doubt concerning the mode of action of diphenylhydantoin (DPH) as an antidysrhythmic drug. A report (Bigger, Bassett & Hoffman, 1968) that DPH did not reduce the maximum rate of depolarization (MRD) in isolated cardiac muscle, except at rather high concentrations, was based on evidence obtained from tissues bathed in solutions containing low potassium concentrations. In solutions containing a normal potassium concentration, however, DPH did reduce MRD (Singh & Vaughan Williams, 1971) at concentrations no higher than those which occur in the blood of treated patients (Bigger, Schmidt & Kutt, 1968), and thus there did not seem to be any reason to suppose that the mode of action of DPH differed greatly from that of many other antidysrhythmic drugs, such as quinidine, procaineamide and lignocaine. In these experiments the effect of Kö 1173 on atrial and ventricular intracellular potentials was studied in Locke solution with the normal potassium concentration. Its effect as a local anaesthetic on frog nerve was also measured.

Methods

Local anaesthesia of nerve

Frog sciatic nerves were stripped of their sheath under magnification, and placed in a three-compartment chamber at room temperature. They were stimulated in moist air at one end, and action potentials were recorded from the other. The segment of nerve in the central compartment was bathed in frog Ringer containing various concentrations of Kö 1173 or of lignocaine. The solution contained (mM): NaCl, 120 ; KCl, 1·88 ; $CaCl_2$, 1·08 ; $NaHCO_3$, 2·38 ; Tris (Sigma) buffer at pH 7·5, 10 ml/litre. The height of the fastest wave of the action potential was measured before and after exposure for 30 min to each concentration of the drug used.

Protection against ouabain-induced arrhythmias

The method used was that described by Vaughan Williams & Sekiya (1963) as modified by Dohadwalla, Freedberg & Vaughan Williams (1969). Guinea-pigs of either sex were anaesthetized with 1·6 g/kg of urethane (i.p.) and were respired artificially. Body temperature was maintained at 37° C by an intrarectal thermistor probe controlling a heated plate under the animal. The electrocardiogram was recorded for 5 s every 2 min and ouabain (3·6 μg) was infused over 30 s from a motor-driven syringe every 2 minutes.

Intracellular potentials

The method was as previously described (Vaughan Williams, 1958 ; Szekeres & Vaughan Williams, 1962). Single fibres were penetrated from the internal surface of isolated rabbit atria suspended horizontally in a bath through which modified Locke solution was recirculated at 32° C by an external oxygenator. Mean values of all parameters were measured according to defined criteria (Vaughan Williams, 1959). Contractions were recorded with an RCA 5734 transducer, and conduction velocity was calculated from the interval between a stimulus (1 ms, strength at least

twice threshold) from an electrode on the left atrium and an action potential recorded from the surface of the right atrium with a bipolar electrode.

Ventricular intracellular potentials were recorded from strips 1·5–2·0 cm in length, 2–3 mm wide, cut from the free wall of the right ventricle. They were stimulated by platinum plates (0·5 × 2·0 cm) fixed parallel to, but not touching, the tissue, one on each side, at sixty/min (duration 2 ms). Atria were stimulated at a frequency about 10% faster than the spontaneous frequency. The solution contained (mM); NaCl, 125; KCl, 5·6; $CaCl_2$, 2·16; $NaHCO_3$, 25; glucose, 11·0; and was gassed with 95% O_2, 5% CO_2, pH 7·4.

The statistical significance of differences was calculated by Student's *t* test.

Drugs used

Atropine sulphate (BDH). Strophanthin-G (Ouabain; BDH). Lignocaine HCl, (Astra-Hewlett). Kö 1173, 1-(2′,6′-dimethyl-phenoxy)-2-amino-propane (Boehringer).

Results

Local anaesthesia

In most recent investigations from this laboratory, procaine HCl was used as the standard local anaesthetic, but in these experiments, because of the resemblance between part of the lignocaine and Kö 1173 molecules, lignocaine was chosen as standard. The results indicated (Table 1) that lignocaine and Kö 1173 were equipotent as local anaesthetics on desheathed frog nerve.

Effects on spontaneous heart rate and on ouabain-induced dysrhythmias in anaesthetized guinea-pigs

Guinea-pigs were anaesthetized with urethane and the ECG was recorded. They were given doses of Kö 1173 of 3 mg (13·86 μmol)/kg (n=6), 6 mg (27·72 μmol)/kg (n=6), or 12 mg (55·44 μmol)/kg (n=5) intravenously. The smaller doses caused an immediate bradycardia (−11·3% and −18·7% respectively), maximal after 2–3 min, but lasting only 5 minutes. Three guinea-pigs were pretreated with 1 mg/kg atropine sulphate (i.v.), but this did not affect the bradycardia observed in response to 3 mg/kg Kö 1173 (i.v.).

The largest dose of Kö 1173 (12 mg/kg) itself caused a temporary dysrhythmia, illustrated in Fig. 2B. An immediate bradycardia was followed by a broadening

TABLE 1. *Local anaesthetic activity of Kö* 1173 *on desheathed frog nerve*

% Reduction of action potential	Lignocaine concentration (mM)	Kö 1173 concentration (mM)
25	0·18±0·025	0·25±0·029
50	0·26±0·029	0·29±0·031
75	0·48±0·027	0·41±0·038

The figures are the means ± S.E. of six experiments, each point having been obtained by interpolation from the log dose-response curves.

of the QRS complex, and loss of sinus control. After 5 min, however, a normal sinus rhythm was fully re-established.

Five minutes after pretreatment with Kö 1173 an infusion of ouabain was started, and the results are presented in Table 2A. The pretreatment had no statistically significant effect on the course of ouabain intoxication. By contrast, in another ten guinea-pigs, an ouabain infusion was started without any pretreatment, and was continued until ventricular fibrillation had commenced, when the infusion was stopped. Kö 1173 (3 mg/ml) was administered slowly intravenously and in every experiment the fibrillation reverted to sinus rhythm, the mean dose required being 3·3 mg (15·3 μmol)/kg (Table 2B). The ouabain infusion was restarted when a stable sinus rhythm had been maintained for 3 minutes. The ventricular fibrillation did not recur in any experiment, and death always occurred in asystole (Fig. 2A). The mean lethal dose of ouabain was significantly higher than in the controls ($P<0{\cdot}01$).

Effects of Kö 1173 on isolated rabbit atria

Kö 1173 had hardly any effect on the spontaneous beating frequency of isolated rabbit atria. The maximum frequency at which they would follow a driving

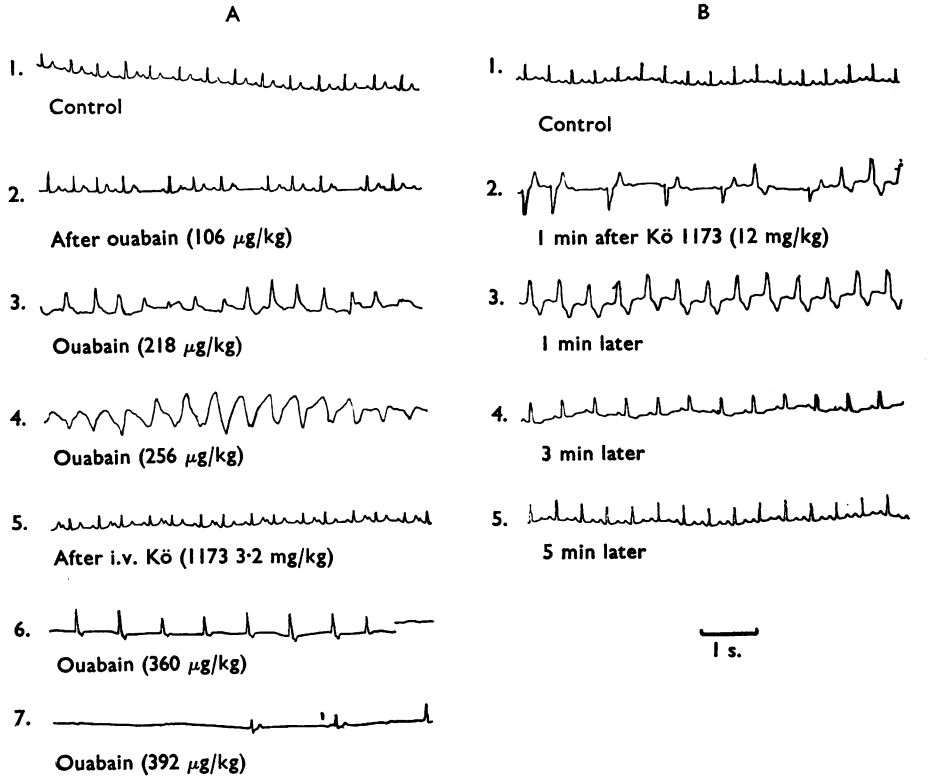


FIG. 2. Effect of Kö 1173 in anaesthetized guinea-pigs. A. Electrocardiogram before (1) and during an intravenous infusion of ouabain. At (4) ventricular fibrillation occurred after the infusion of 256 μg (351 nmol)/kg. The infusion was stopped and Kö 1173 given intravenously. Sinus rhythm was restored after 3·2 mg (14·85 μmol)/kg had been administered (5). After 3 min of stable sinus rhythm the ouabain infusion was continued, until the heart stopped. B. Effect of 12 mg (55·44 μmol)/kg of Kö 1173 (i.v.) on the electrocardiogram. Similar results were observed in all the five animals given this dose.

stimulus, however, was significantly reduced. Conduction velocity was retarded and electrical threshold raised. The amplitude of contractions was considerably reduced. All these results have been summarized in Table 3, and for greater simplicity only differences in excess of 1% have been recorded.

TABLE 2. *Effect of Kö* 1173 *on ouabain-induced cardiac dysrhythmias in guinea-pigs*

	Mean (±S.E.) amount of ouabain (nmol/kg i.v.) required to produce				
	Unequal R-R interval	Ventricular ectopic beats	Ventricular rhythm	Ventricular fibrillation	Cardiac arrest
A. Control	120± 7	281±14	310±16	331±16 (29/30)	429±17
B. After pretreatment with Kö 1173 (i.v.)					
Dose of Kö 1173 (μmol/kg)					
13·86	145± 9	250±12	341±23	361±22 (6/6)	452±26
27·72	135±14	297±14	327±17	354±19 (5/6)	414±25
55·44	141±18	266±13	284±22	311±23 (5/5)	396±23
C. Without pretreatment (2nd control series)					
	136± 8	314±13	328±14	344±21 (10/10) All converted by mean dose of 15·3 μmol/kg Kö 1173	532±16

TABLE 3. *Effects of Kö* 1137 *on the spontaneous frequency, maximum driven frequency, electrical threshold, conduction velocity and contractions of isolated rabbit atria*

Effect measured	Concentration Kö 1173 (μM)	Duration of exposure to drug (min) 10	30	60	120
Spontaneous frequency	23·1	−2·5	−3·6	−3·6	−3·5
Maximum driven frequency	13·86	−4·8	−13·9	−25·8	−29·8
	23·1	−12·7	−32·1	−47·8	−50·3
Electrical stimulus threshold	13·86	+9·1	+19·4	+29·4	+33·7
	23·1	+13·9	+26·7	+42·6	+55·7
Conduction velocity	13·86	−12·8	−18·7	−31·7	−33·9
	23·1	−14·3	−29·6	−39·8	−46·7
Contraction amplitude	13·86	−11·3	−19·4	−27·9	−31·4
	23·1	−18·4	−27·8	−34·7	−41·7

The effects of concentrations of 4·62 μM (1 mg/l.), 13·86 μM (3 mg/l.) and 23·1 μM (5 mg/l.) were studied, but the results have not been given unless the changes from control values exceeded 1%. The figures are the means of three experiments, and indicate changes as percentage of control. For simplicity, the S.E.s have been omitted from the table, since the variation was small, seldom more than 5%.

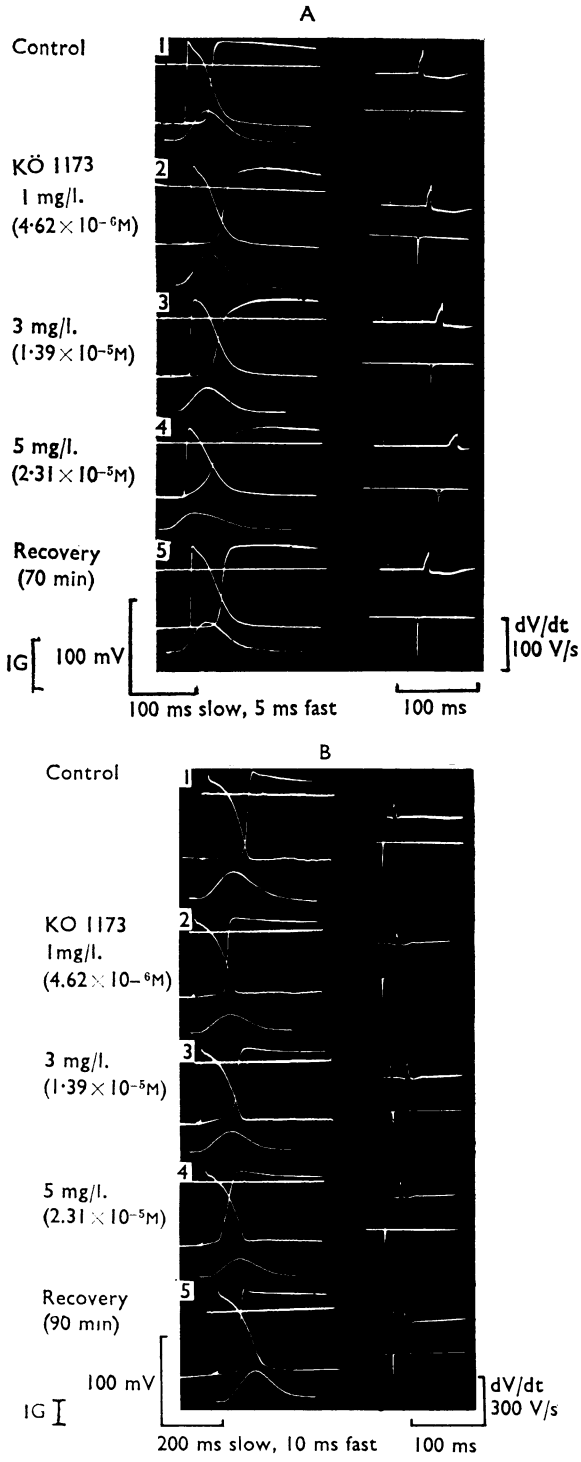


FIG. 3. Effect of various concentrations of Kö 1173 on intracellular atrial (A) and ventricular (B) potentials of isolated rabbit heart muscle. In each frame the oscilloscope traces represent: Left, horizontal trace; zero potential with the microelectrode in the external solution. Middle traces, intracellular potentials at slow and fast sweep speeds. Lowest trace, contraction. Right, upper trace; stimulus artifact from an electrode on left atrium and action potential recorded with a bipolar electrode on the surface of the right atrium. Lower trace, differentiated record of the intracellular potential, the depth of the spike being proportional to MRD.

Effect of Kö 1173 on intracellular potentials in isolated atrial and ventricular muscle

The effects of various concentrations of Kö 1173 on atrial and ventricular intracellular potentials are depicted in Fig. 3 A and B respectively. It was evident that in both tissues Kö 1173 had a typical 'class 1' antidysrhythmic action (Vaughan Williams, 1970), defined as a depression of the maximum rate of depolarization (MRD) and overshoot potential, in the absence of any fall in resting potential or significant prolongation of the duration of the action potential. The action of Kö 1173 was rapid, the maximal effect of each concentration being apparent within 20–30 minutes. Similarly, control values were fully restored on washing out the drug, even after exposure to the highest concentration. The results are summarized in Table 4.

Discussion

In a recent paper (Singh & Vaughan Williams, 1971) it was concluded that there was no reason to suppose that lignocaine and diphenylhydantoin (DPH) had a mode of action on cardiac muscle greatly different from that of many other antidysrhythmic drugs with local anaesthetic properties. At a normal external potassium concentration they depressed the maximum rate of depolarization (MRD) of atrial and ventricular intracellularly recorded action potentials, and reduced the overshoot potential, without altering the resting potential or the duration of the phase of repolarization. This is not to imply that there are no differences between DPH, lignocaine and other drugs with such 'class 1' actions (Vaughan Williams, 1970; Singh & Vaughan Williams, 1971), but that if there are differences they may be attributable to extracardiac factors, or to variations in the relative sensitivity of atrial, ventricular or Purkinje tissue, rather than to any fundamental difference in their mode of action on the cardiac membrane.

In this context the new compound Kö 1173 was of particular interest, both because it had been found in animal experiments to resemble DPH in its anticonvulsant potency, and because in its chemical structure it bore some resemblance

TABLE 4. *Effect of Kö 1173 on intracellular potentials*

	Conc. (μM)	No. fibres	Resting potential (mV)	Action potential (mV)	MRD (V/s)	APD_{50} (ms)	APD_{90} (ms)
A. Rabbit atrium							
Kö 1173	0	23	62·7±1·1	86·9±1·4	93·4±2·8	53·8±1·8	107·6±1·7
	4·6	31	63·8±1·0	84·6±1·2	78·1±3·1*	54·7±1·2	103·7±1·8
	13·9	27	61·9±0·8	79·7±1·3*	51·3±2·9†	52·8±1·0	104·8±1·2
	23·1	21	61·7±1·1	75·4±1·2†	40·6±2·7†	55·8±2·1	104·9±1·3
Recovery	0	16	64·1±1·7	90·1±1·8	98·7±3·2	54·7±1·7	105·7±2·1
B. Rabbit ventricle							
Control	0	24	81·7±2·1	98·7±3·1	302·4±4·8	152·7±2·8	186·7±2·7
	4·6	18	84·7±3·1	90·3±2·9*	270·2±3·4*	155·7±2·3	180·7±3·1
	13·9	28	80·8±2·3	87·4±1·7†	190·3±2·7†	150·8±1·8	180·2±3·0
	23·1	19	80·7±1·7	83·7±2·1†	140·2±2·7†	152·7±2·1	181·6±2·7
Recovery	0	16	83·7±1·9	100·2±3·1	316·2±3·1	155·8±1·7	180·8±2·0

Statistical significance of difference from control: * $P<0.01$; † $P<0.001$.
The results are means ± S.E. from four experiments each. The concentration of Kö 1173 was increased by cumulative addition, allowing 40–60 min for equilibration with each increment. The last two columns give the duration of the action potential from its peak until repolarization to 50% (APD_{50}) or to 90% (APD_{90}) of the diastolic potential.

to lignocaine. We have found that its local anaesthetic activity on frog nerve is equal to that of lignocaine (that is twice that of procaine). In isolated rabbit atria Kö 1173 had little effect on the spontaneous frequency, but reduced the maximum frequency at which they would follow a stimulus. It also reduced conduction velocity and contraction amplitude, and raised the electrical threshold. It had a typical 'class 1' action on intracellular potentials in both atrial and ventricular muscle, that is it depressed MRD and overshoot, without altering the resting potential or the duration of the action potential.

Perhaps the most interesting action of Kö 1173, however, was its effect on ouabain-induced dysrhythmias. In anaesthetized guinea-pigs in which ventricular fibrillation had been produced by an intermittent intravenous infusion of ouabain, the administration of Kö 1173 caused reversion to sinus rhythm in ten out of ten animals, the mean intravenous dose required being between 1·8–4·9 mg/kg, mean 3·3 mg/kg (15·3 μmol/kg). When the infusion of ouabain was continued, ventricular fibrillation did not recur, and the lethal dose of ouabain was significantly increased. These results on guinea-pig confirm the effects of Kö 1173 on established ouabain-induced dysrhythmias in dogs reported by Allen, Kofi Ekue, Shanks & Zaidi (1970). The latter authors, however, quoted a personal communication that Kö 1173 did not have local anaesthetic activity.

In contrast with these results, pretreatment even with a dose of Kö 1173 as high as 12·0 mg (55·44 μmol)/kg had no significant effect on the toxicity of a subsequent ouabain infusion, with the implication that the *in vivo* action of Kö 1173 is very short-lived. Its effect on electrical threshold, conduction velocity and contractions in isolated atria exposed to a constant concentration of the drug continued to increase during an hour or more, however, so that the brevity of its *in vivo* action is presumably due to a rapid disappearance from the circulation.* The prediction that might be made from these findings is that, *in vivo*, Kö 1173 could be an effective and safe (because of its brief action) antidysrhythmic drug, delivering a sharp chemical shock, as it were, analogous to an electrical defibrillation.

* Note added after submission of MS. Blood concentrations of Kö 1173 have now been measured in rats (personal communication, P. A. Knowlson, Boehringer Ltd.). After 10 mg/kg (i.v.) the mean (n=12) blood concentration was 2 μg/ml after 12 min, and fell to 1 μg/ml after a further 31 min, and was halved again after another 44 minutes.

B. N. S. is a Nuffield Dominions Demonstrator (New Zealand).

REFERENCES

ALLEN, J. D., KOFI EKUE, J. M., SHANKS, R. G. & ZAIDI, S. A. (1970). The effect on experimental cardiac arrhythmias of a new anticonvulsant agent, Ko 1173, and its comparison with phenytoin and procainamide. *Br. J. Pharmac.*, **39**, 183–184*P*.

BIGGER, T., BASSETT, A. L. & HOFFMAN, B. (1968). Electrophysiological effects of diphenylhydantoin on canine Purkinje fibers. *Circulation Res.*, **22**, 221–236.

BIGGER, J. T., SCHMIDT, D. N. & KUTT, H. (1968). Relationship between the plasma level of diphenylhydantoin sodium and its cardiac anti-arrhythmic effects. *Circulation*, **38**, 363–374.

DOHADWALLA, A. N., FREEDBERG, A. S. & VAUGHAN WILLIAMS, E. M. (1969). The relevance of β-receptor blockade to ouabain-induced cardiac arrhythmias. *Br. J. Pharmac.*, **36**, 257–267.

DREIFUS, L. S. & WATANABE, Y. (1970). Current status of diphenylhydantoin. *Am. Heart J.*, **80**, 709–713.

SINGH, B. N. & VAUGHAN WILLIAMS, E. M. (1971). The effect of lignocaine and diphenylhydantoin on the electrophysiological properties of atrial and ventricular muscle in solutions containing normal and low potassium concentrations. *Circulation Res.*, **29**, 286–296.

SZEKERES, L. & VAUGHAN WILLIAMS, E. M. (1962). Antifibrillatory action. *J. Physiol., Lond.*, **160**, 470–482.

VAUGHAN WILLIAMS, E. M. (1958). The mode of action of quinidine in isolated rabbit atria interpreted from intracellular records. *Br. J. Pharmac. Chemother.*, **13**, 276–287.

VAUGHAN WILLIAMS, E. M. (1959). The effect of changes in extracellular potassium concentration on the intracellular potentials of isolated rabbit atria. *J. Physiol., Lond.*, **146**, 411–421.

VAUGHAN WILLIAMS, E. M. (1970). Classification of anti-arrhythmic drugs. In: *Symposium on Cardiac Arrhythmias*, ed. Sandhøe, E., Flensted-Jensen, E. & Olesen, K. H., pp. 449–472. Sweden: Abastra Södertälje.

VAUGHAN WILLIAMS, E. M. & SEKIYA, A. (1963). Prevention of arrhythmias due to cardiac glycosides by block of β-sympathetic receptors. *Lancet*, **i**, 420–421.

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