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The effect of prolonged treatment with oxyfedrine on intracellular potentials and on other features of cardiac function in rabbits and guinea-pigs

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Summary

1. Previous work in acute experiments has shown that the main pharmacological action of oxyfedrine is stimulation of β-adrenoceptors, yet there have been clinical reports that the drug is beneficial in the treatment of angina pectoris.

2. In the present experiments rabbits and guinea-pigs were treated for several weeks with daily i.p. injections of oxyfedrine.

3. A daily dosage of 15 mg/kg oxyfedrine had no effect on growth rate for 4 weeks, but thereafter the growth rate of treated animals fell below that of controls.

4. The heart weights of the treated animals, expressed as a percentage of body weight, were significantly lower than those of controls.

5. Measurement of intracellular potentials in hearts taken from treated rabbits showed that the main effects were a reduction in the maximum rate of depolarization and a prolongation of the plateau of the action potential.

6. Guinea-pigs treated for 6 weeks with 15 mg/kg oxyfedrine daily i.p. were protected to some extent from the toxic effect of ouabain infused intravenously.

Introduction

The mode of action of drugs on cardiac muscle can only be interpreted in terms of what is known of the biophysics of the normal tissue. Although it has been possible to make modifications of Hodgkin–Huxley equations which can give reproductions of the action potentials of Purkinje fibres (Noble, 1962), evidence that the required voltage-current relations actually exist in cardiac muscle has been lacking, and no satisfactory computation of action potentials based on the experimental data available has so far been possible. Not only are more currents involved than in nerve, including inward calcium currents during the plateau (Beeler & Reuter, 1970) and outward currents responsible for repolarization (Noble & Tsien, 1969) and for pacemaking activity (Hauswirth, Noble & Tsien, 1968), but cardiac potentials are more labile, and subject to influence by hormones and drugs. Differences in thyroid state, for example, have a profound effect upon the duration of the plateau (Freedberg, Papp & Vaughan Williams, 1970), and the possibility has to be considered that currents associated with active processes involved in contraction or ion-pumping by the membrane may contribute currents which partly determine the shape of the normal potential.

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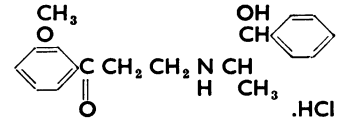

FIG. 1. Structure of oxyfedrine.

In this context it may be noted that an anti-anginal drug amiodarone, and a β-adrenoceptor blocking drug, MJ 1999, have been shown to alter the duration of the plateau of cardiac action potentials (Singh & Vaughan Williams, 1970a and b). Oxyfedrine, L-3-methoxy-ω-(1-hydroxy-1-phenylisopropylamino)-propiophenone hydrochloride (Fig. 1) has been used recently in Germany and Belgium for the treatment of patients with angina pectoris. Since the main acute pharmacological effect of this drug is that of an agonist on β-adrenoceptors, there seemed to be no obvious reason why it should be effective in angina ; on the contrary, one might have expected it to be deleterious. Nevertheless clinical reports have appeared attesting to its efficacy in angina pectoris (Greif & Liertzer, 1967 ; Stampfer, 1969 ; Raisp, 1970 ; Carlier, 1970 ; Marner, 1970). It seemed of interest to determine what would be the electrophysiological and other cardiac effects of prolonged treatment with this drug, with the possibility in mind that acute and chronic actions might be different.

Methods

The rabbits used were New Zealand Whites, bred and reared under strict barrier conditions at the Geigy Toxicology Department at Macclesfield. Litters of four rabbits were selected 2 weeks after birth. Three rabbits in each litter received daily i.p. injections of oxyfedrine, the fourth received daily injections of vehicle. All animals were kept at 20° C ($\pm$2° C) and r.h. 50–70%. Food (irradiated Styles Oxoid Rabbit Diet) and sterile filtered water were available to the does and litters *ad libitum*. After a period of several weeks treatment the rabbits were delivered to Oxford, where the same dose regime was maintained. They were used for experiment within two or three days of their arrival, in all cases 16–24 h after the last dose of oxyfedrine given.

Guinea-pigs were similarly reared, treated and delivered to Oxford for experimental study.

Electrocardiogram

Attempts were made to take the electrocardiogram on unanaesthetized rabbits by means of electrodes held by light springs to patches of clipped and moistened skin on the limbs, but it proved impossible to maintain the animals quiescent. The rabbits were therefore anaesthetized with ether and as soon as they were unconscious, needle electrodes were inserted under the skin. The electrocardiogram was recorded at 1 min intervals until the rabbits recovered from the anaesthetic (usually 5–8 min). During the 3–4 min before they started moving, they lay quietly and the heart rate remained stable within a few beats/minute. The mean of the recordings during this quiescent recovery period was taken as the '*in vivo* spontaneous heart rate'. The rabbits were then killed by a blow on the head, their hearts were removed, and the atria dissected in oxygenated Locke solution.

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 a pair of Ag-AgCl electrodes on the left atrium and an action potential recorded from the surface of the right atrium with a platinum bipolar electrode.

The solution contained (mM): NaCl, 125 ; KCl, 5·6 ; $CaCl_2$, 2·16 ; $NaHCO_3$, 25 ; glucose, 11·0 ; and was aerated with 95% O_2, 5% CO_2 ; pH was 7·4.

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

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 urethane i.p. and were respired artificially. Body temperature was maintained at 37° C by 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.

Drugs used

Ethyl carbamate (B.D.H.) ; strophanthin G (ouabain, B.D.H.) ; oxyfedrine hydrochloride (Geigy). The aqueous vehicle in which the oxyfedrine was dissolved (10 mg/ml) contained per ml, 150 mg of 96% alcohol, 250 mg of propylene glycol, and 0·003 ml of 25% HCl.

Results

Effect of oxyfedrine on growth

New Zealand White rabbits have a fast growth rate, reaching over 40 g/day, which may be compared with a mean growth rate of 35 g/day in mongrel rabbits of the same age previously reared in this laboratory. For the first four weeks of treatment oxyfedrine had no effect on the rate of growth of the young rabbits. Six litters were treated, three animals in each litter receiving 15 mg/kg in 1·5 ml vehicle daily i.p. for 30 days ; the fourth received daily injections of 1·5 ml/kg vehicle without drug. Each animal was weighed daily, but for simpler presentation the growth rates have been averaged over 3 day periods. The mean growth rates during the first four weeks of treatment of the treated and the control animals have been plotted in Fig. 2A.

After four weeks the treated animals were divided into two groups. Group 1 (12 rabbits from 4 litters) continued to receive the same treatment as before, 15 mg/kg oxyfedrine daily i.p. In group 2 the dose was raised to 40 mg/kg daily. Although both treated groups continued to gain weight, the rate of growth fell below that of the controls (Fig. 2B).

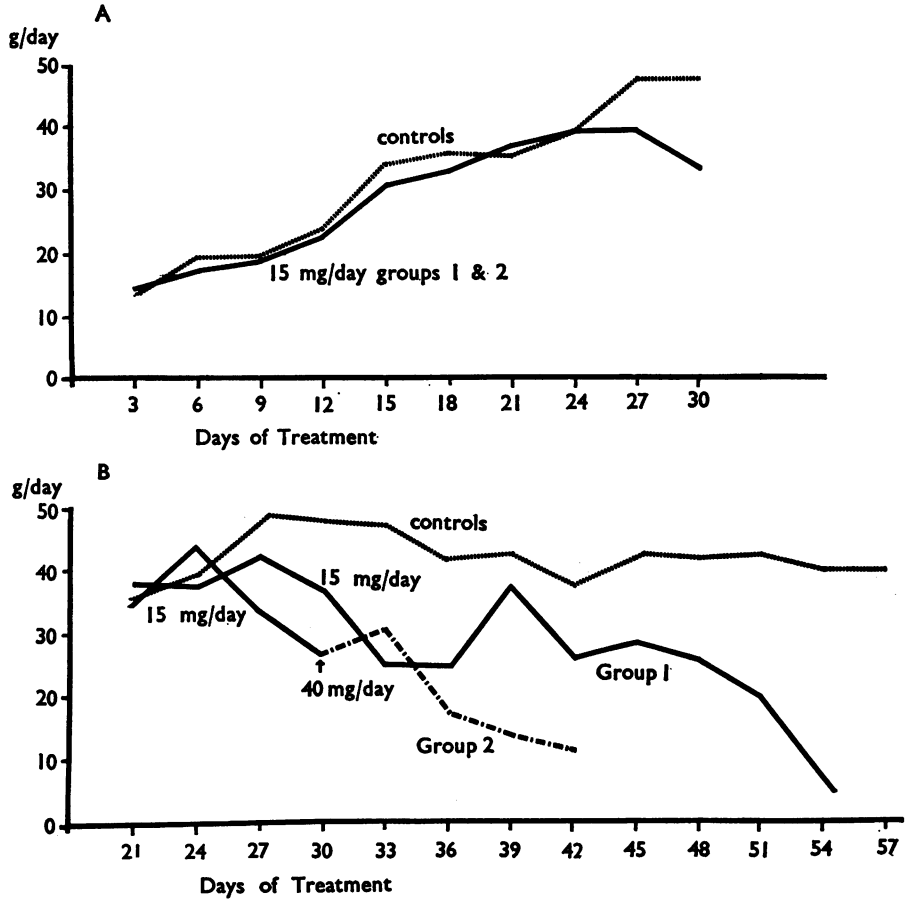

FIG. 2. Effect of oxyfedrine on the growth rate of young rabbits. Treatment began when they were two weeks old. Ordinates: mean growth rate in g/day. Abscissae: number of days since start of treatment.

The spontaneous heart rate of the New Zealand White rabbits was much higher four controls from the same litters. The group 2 rabbits were killed after a total of 6 weeks treatment (i.e. four weeks at 15 mg/kg, two weeks at 40 mg/kg). The mean wet weight of the hearts of the control animals, expressed as a percentage of the total body weight, was $0{\cdot}2652 \pm 0{\cdot}0086\%$ (mongrel rabbits 0·2353%). The mean heart weight of the treated animals in group 1 was $0{\cdot}2287 \pm 0{\cdot}0023\%$, which was highly significantly different from the controls (−14%, $t=5{\cdot}37$). In group 2 the mean heart weight was 10·7% less than the control. Taking groups 1 and 2 together the overall mean heart weight was $0{\cdot}2342 \pm 0{\cdot}0031\%$ of the body weight, a highly significant reduction of 11·5% ($t=4{\cdot}28$). In mongrel rabbits six weeks after thyroidectomy the heart weights, as a proportion of body weight, were 21·5% less than those of euthyroid controls (Freedberg *et al.*, 1970).

Effects on heart rate

The spontaneous heart rate of the New Zealand White rabbits was much higher than that of mongrel rabbits of the same age, which are commonly in the range 200–240 beats/minute. The mean *in vivo* spontaneous heart rate of the control

N.Z. rabbits was 374 ± 28 beats/minute. The control rabbits received daily injections of vehicle, and the possibility had to be considered that either the vehicle or the daily disturbance might have had some effect, for example, on thyroid state. Four New Zealand rabbits of the same age as the controls, but which had received no injections, were studied. There was no difference between them and the injected controls, either in heart rate, or any other parameter of cardiac function measured.

In the treated rabbits the mean spontaneous *in vivo* heart rate was 324 ± 9 beats/min in group 1, and 287 ± 27 in group 2. The overall mean of both groups was 315 ± 10 beats/minute. The difference of −59 beats/min from the controls was statistically significant. The electrocardiogram showed also that the Q-T interval was 9% longer in the treated animals than in the controls. Although this difference was not statistically significant, it was of interest in the light of the results of the intracellular recordings of action potentials subsequently obtained.

Isolated atria

In the isolated organ bath at 32° C the mean spontaneous frequency of the treated atria was slightly less than that of the controls (−6 beats/min), as was the maximum frequency at which a stimulus could be followed (−36 beats/min), but neither difference reached statistical significance. There was no difference in electrical threshold or conduction velocity, in contrast to the effect of high concentrations of oxyfedrine in acute experiments (Papp & Szekeres, 1972).

Intracellularly recorded potentials

There was no difference ($t<1$ for all measurements) between the records obtained from the treated rabbits in groups 1 and 2, and so the results from both groups have been pooled and compared with those from the controls (Table 1). Recordings were made from 10–20 different fibres in each rabbit. One animal in group 2 died before the completion of treatment, and recordings were not obtained from one rabbit in group 1 because of a power cut.

TABLE 1. *Effect of oxyfedrine on cardiac intracellular potentials*

	Number of rabbits	Resting potential		Action potential		Maximum rate of depolarization		50% Repolarization time		90% Repolarization time	
		mV	Diff.	mV	Diff.	V/s	Diff.	ms	Diff.	ms	Diff.
Controls	6	77·4 ±0·2		106·2 ±1·4		127·3 ±8·9		60·5 ±3·3		96·7 ±4·1	
Treated Groups 1 and 2	16	74·2 ±0·9	−3·2*	100·0 ±1·3	−6·2†	98·4 ±5·5	−28·9†	76·7 ±2·4	+16·2‡	113·8 ±3·0	+17·1‡

Statistical significance: * $P<0{\cdot}05$; † $P<0{\cdot}02$; ‡ $P<0{\cdot}01$.

The resting and action potentials of the atrial fibres in the treated animals were a few mV less than those of the controls. Although the differences were statistically significant, they were comparatively trivial (−4% and −6% respectively). There was a much larger reduction (−23%) in the maximum rate of depolarization. The most highly significant change was a large prolongation of the ' plateau ' (50% repolarization time) of the action potential (+27%) (Fig. 3).

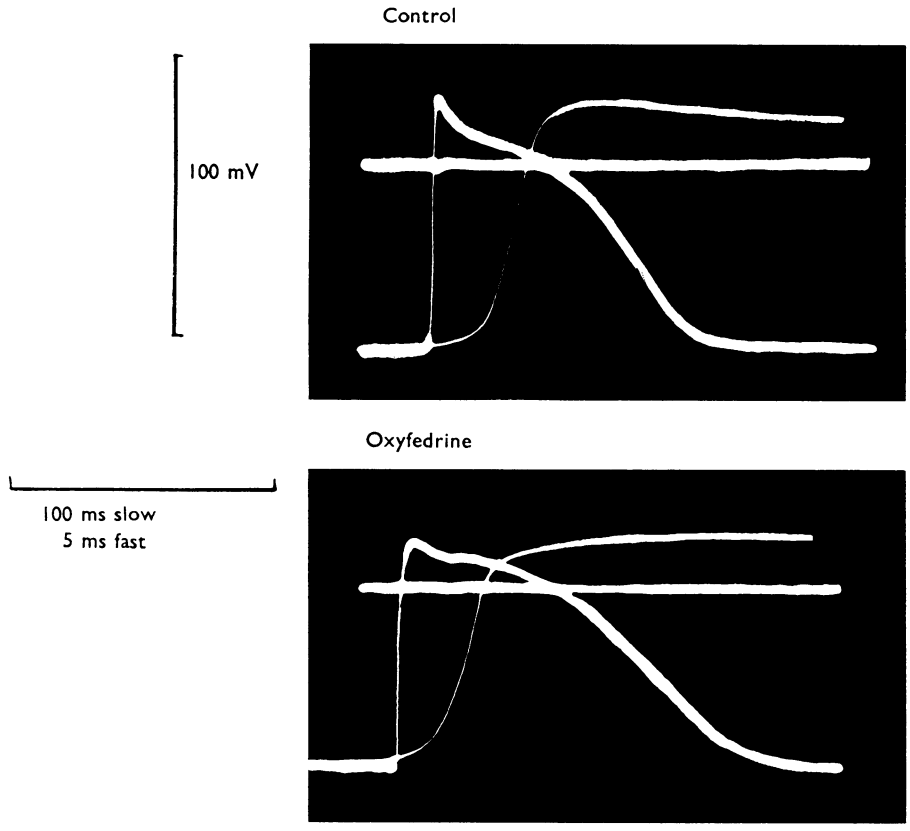

FIG. 3. Effect of treatment with oxyfedrine 15 mg/kg daily for six weeks on rabbit atrial potentials. In each panel: horizontal trace, zero potential; other traces, intracellular potential at slow and fast sweep speeds. The control potentials were recorded from atria taken from a litter mate given daily injections of solvent for six weeks.

Ouabain toxicity in guinea-pigs

A prolongation of the cardiac action potential would automatically prolong the absolute refractory period, and this should have an antidysrhythmic effect. Two groups of guinea-pigs were treated, one being given daily i.p. injections of 15 mg/kg oxyfedrine for six weeks, the other receiving 40 mg/kg for four weeks. They were anaesthetized with urethane and the electrocardiogram was recorded, at 2 min

TABLE 2. *Effect of oxyfedrine (15 mg/kg daily for 42 days) on ouabain-induced dysrhythmia. Mean doses of ouabain (in μg/kg) which produced a, b, c, d, and e are indicated*

	Number of guinea-pigs	Mean weight	a Irregular sinus rhythm	Diff.	b Ventricular ectopic beats	Diff.	c Persistent ventricular tachycardia	Diff.	d Ventricular flutter/fibrillation	Diff.	e Cardiac arrest	Diff.
Controls	10	502 g ± 24·4	60·3 ± 14·0		121·0 ± 6·1		159·8 ± 11·0		202·0 ± 15·1		271·5 ± 18·6	
Treated	8	486 g ± 17·5	119·3 ± 18·7	+ 59*	173·8 ± 20·1	+ 52·8*	218·0 ± 36·8 (t = 1·68)	+ 58·2	280·6 ± 38·9 (t = 2·06)	+ 78·6	334·6 ± 38·5 (t = 1·59)	+ 63·1

Statistical significance: * $0{\cdot}02 > P > 0{\cdot}01$

intervals, and also observed continuously on an oscilloscope. An intermittent i.v. infusion of ouabain from a motor-driven syringe was given, and the amounts infused were noted when the following changes occurred: (a) irregular sinus rhythm; (b) ventricular extrasystoles; (c) complete atrio-ventricular block with persistent ventricular tachycardia; (d) ventricular flutter-fibrillation; (e) cardiac arrest. The results of the experiments on the first group of guinea-pigs (15 mg/kg for 6 weeks) are presented in Table 2. The controls were given daily intraperitoneal injections of 1·5 ml/kg of vehicle without drug.

The amount of ouabain required before ventricular ectopic beats appeared was significantly higher in the treated than the control animals. Although the differences in the lethal doses and in those producing ventricular fibrillation were below statistical significance the trend towards protection was clear.

In the second group of animals which were given 40 mg/kg oxyfedrine daily, only half survived for four weeks, and the mean weight of the survivors was over 20% less than that of the controls, so the results are not presented.

Discussion

Perhaps the main point of interest in the results described here is that a drug may have quite different effects after chronic administration from those apparent when it is given acutely. This is particularly important for compounds used in the treatment of angina pectoris, since they may be given regularly for months or even years. In acute experiments oxyfedrine is a β-adrenoceptor agonist, with effects similar to those of isoprenaline. In concentrations up to 10 mM it produces dose-related increases in heart-rate and force of contraction in isolated rabbit atria; concentrations of 100 mM and more depressed both rate and force of beat, but such concentrations could have little relevance to the clinical use of the drug (Papp & Szekeres, 1972). Similar agonist activity was observed on conduction velocity and maximum driven frequency, which were increased by the lower concentrations, although depressed by very high concentrations. The agonist effects were blocked by LB 46, 4-(2-hydroxy-3-isopropylaminopropoxy)-indole, a β-adrenoceptor blocking drug about 20 times more potent than propranolol. In addition, it was found that oxyfedrine was a local anaesthetic on frog nerve, with 3·4 times the activity of procaine (Papp & Szekeres, 1972).

In another series of acute experiments with oxyfedrine, Homburger & Antoni (1972) observed by means of intracellular recording from cardiac fibres not only the β-adrenoceptor stimulating agonist action of oxyfedrine, but also a direct membrane depressant, 'class I' action (Vaughan Williams, 1970; Singh & Vaughan Williams, 1971) or quinidine-like action, common to anti-arrhythmic drugs with local anaesthetic properties. In fibres in which the resting potential had been depressed by raising the external potassium to 17 mM, oxyfedrine 2 mg/l. ($5·7 \times 10^{-6}$M) *increased* the resting potential and the maximum rate of depolarization (MRD) of the action potential, presumably by stimulation of β-adrenoceptors (Homburger & Antoni, 1972). Exposure to ten times this concentration in normal solution for 30–50 min depressed contractions and MRD, with very little effect on resting potential or the duration of the action potential; thus oxyfedrine had the 'class I' action on cardiac muscle typical of a local anaesthetic.

In contrast to these acute effects, daily administration of oxyfedrine for six weeks or more had no significant effect on electrical threshold, on conduction

velocity, or on the maximum frequency at which isolated atria could be driven. There was a large prolongation (+27%) of the duration of the 'plateau' of the intracellularly recorded cardiac action potential. A prolongation of the mean Q-T interval in the electrocardiogram of the treated animals was also observed, and in view of the intracellular findings, was probably real although not statistically significant. Delayed repolarization would prolong the absolute refractory period, and prolonged administration of oxyfedrine should, therefore, have an anti-dysrhythmic effect. Six weeks' treatment with 15 mg/kg daily caused a significant protection of guinea-pigs against ouabain-induced ectopic ventricular beats.

Oxyfedrine has been reported to be beneficial to patients with angina pectoris. It is not easy to see why such an effect should be produced by a drug which has *agonist* activity on β-adrenoceptors, increasing cardiac oxygen consumption and the force of contractions (Himms-Hagen, 1972). *Prima facie* such actions might be expected to precipitate angina rather than relieve it. When the effect of long-term administration is considered the anti-anginal action is more easily explained, because a similar prolongation of the action potential occurs after thyroidectomy (Freedberg *et al.*, 1970), and after 6 weeks' treatment with another anti-anginal drug, amiodarone (Singh & Vaughan Williams, 1970a).

This is not to imply that these compounds have an antithyroid action. Amiodarone did not affect the weight of the thyroid gland, and patients treated with amiodarone for periods of years have not shown signs of hypothyroidism. However, it does seem possible that the drugs produce some circulatory changes or have other cardiac effects such that the work-load on the heart is reduced, as it is in hypothyroidism, and that the prolongation of the action potential is a reflection of some intracellular readjustment to the reduced load.

If this hypothesis is correct, measurement of the duration of the action potential after prolonged administration of a drug could serve as a useful test for its anti-anginal activity. It will clearly be of interest to observe the effects of prolonged administration of β-adrenoceptor blocking drugs on the cardiac action potential, in the hope of finding a clue to the so far unexplained beneficial effects, seen only after several weeks of treatment, of propranolol in hypertension. Such studies are in progress.

Some further observations support the view that a secondary response to oxyfedrine appears after a few weeks of treatment. For the first four weeks of treatment with 15 mg/kg daily i.p. oxyfedrine had no effect on the growth rate of young rabbits, but thereafter, although the animals still gained weight, they did so at a slower rate than the controls. The mean heart weight of the treated animals, expressed as a percentage of their total weight, was 11·5% less than that of the controls. In previous experiments, the heart weights of rabbits six weeks after thyroidectomy were 21·5% less than those of euthyroid controls.

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