Br. J. Pharmac. (1968), **34**, 43–55.

The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias

A. M. BARRETT AND VALERIE A. CULLUM

Pharmacological Research Department, Pharmaceuticals Division, Imperial Chemical Industries Limited, Alderley Park, Macclesfield, Cheshire

1. The optical isomers of propranolol have been compared for their β-blocking and antiarrhythmic activities.

2. In blocking the positive inotropic and chronotropic responses to isoprenaline, (+)-propranolol had less than one hundredth the potency of (−)-propranolol. At dose levels of (+)-propranolol which attenuated the responses to isoprenaline, there was a significant prolongation of the *PR* interval of the electrocardiogram.

3. The metabolic responses to isoprenaline in dogs (an increase in circulating glucose, lactate and free fatty acids) were all blocked by (−)-propranolol. (+)-Propranolol had no effect on fatty acid mobilization but significantly reduced the increments in both lactate and glucose.

4. Both isomers of propranolol possessed similar depressant potency on isolated atrial muscle taken from guinea-pigs.

5. The isomers of propranolol exhibited similar local anaesthetic potencies on an isolated frog nerve preparation at a level approximately three times that of procaine. The racemic compound was significantly less potent than either isomer.

6. Both isomers of propranolol were capable of preventing adrenaline-induced cardiac arrhythmias in cats anaesthetized with halothane, but the mean dose of (−)-propranolol was 0.09 ± 0.02 mg/kg whereas that of (+)-propranolol was 4.2 ± 1.2 mg/kg. At the effective dose level of (+)-propranolol there was a significant prolongation of the *PR* interval of the electrocardiogram. Blockade of arrhythmias with both isomers was surmountable by increasing the dose of adrenaline.

7. Both isomers of propranolol were also capable of reversing ventricular tachycardia caused by ouabain in anaesthetized cats and dogs. The dose of (−)-propranolol was significantly smaller than that of (+)-propranolol in both species but much higher than that required to produce evidence of β-blockade.

8. The implications of these results are discussed.

Pronethalol is a specific competitive antagonist of adrenoceptive β-receptors and the (−) isomer has been shown to be 40 times as active as the (+) isomer

(Howe, 1963). It also has antifibrillatory activity greater than that of quinidine (Sekiya & Vaughan Williams, 1963), and has been shown to be a local anaesthetic twice as potent as procaine (Gill & Vaughan Williams, 1964). Local anaesthetics are not usually stereospecific (Ariëns, 1967), and the observations of Lucchesi (1964, 1965), that (+)-pronethalol and (±)-pronethalol had similar antifibrillatory activity, support the view that antiarrhythmic properties are not associated with blocking activity at adrenergic β-receptors. In a comparison of the optical isomers of the similar compound, propranolol (Howe & Shanks, 1966), it was again found that the (−) isomer was the more active β-receptor antagonist but, in contrast to the (+) isomer, it was virtually incapable of counteracting ouabain-induced ventricular tachycardia. Preliminary experiments showed that both isomers had similar local anaesthetic potency and the present work describes further tests of their β-blocking and local anaesthetic properties and antiarrhythmic activity to see how far these may be associated.

Methods

Blockade of β-receptors

The animals used were male rats (200–220 g), from the specific pathogen free colony bred at Alderley Park, and mongrel dogs of either sex, weighing 11–14 kg. The rats were anaesthetized with pentobarbitone (55 mg/kg, intraperitoneally). Propranolol or one of its isomers was given subcutaneously in 0.9% NaCl solution in volumes of 0.1 ml./100 g 30 min before challenge by intravenous isoprenaline (0.1 μg/kg). Heart rates were recorded using a cardiotachometer (Horsfall, 1965). Twelve rats were used in each group for these experiments. For the measurement of cardiovascular responses, dogs were anaesthetized with intravenous thialbarbitone (30 mg/kg), followed by chloralose (60 mg/kg). The techniques have been described previously (Dunlop & Shanks, 1968). For the measurement of metabolic responses, dogs, fasted for 20 hr, were anaesthetized with pentobarbitone (30 mg/kg), supplemented by additional anaesthetic when necessary. Blood samples were withdrawn from the abdominal aorta by means of a polythene cannula inserted through the left femoral artery. No heparin was used and clotting was reduced by keeping the cannula full of sterile saline. Samples (5 ml.) of blood were withdrawn at 10-min intervals immediately before and during a response and at 30-min intervals during recovery periods. Not more than 100 ml. was taken from any dog. After a 30-min stabilization period, dogs were infused intravenously with isoprenaline (0.5 μg/kg/min) for 10 min. Three further infusions of isoprenaline were given at 90-min intervals. Fifteen minutes before the third isoprenaline infusion, an intravenous infusion of saline, (−)-propranolol or (+)-propranolol was commenced and continued until the end of the experiment. Three dogs were used in each group in these experiments. Biochemical determinations were as previously described (Barrett & Thorp, 1968).

Negative inotropic activity

Left atria from guinea-pigs (400–600 g) were studied by the method of Blinks (1965). The concentration producing 50% depression of the original force of contraction was determined graphically.

Local anaesthetic activity

This was measured as described by Dunlop & Shanks (1968). Five nerves were used to determine the effect of each concentration of propranolol.

Adrenaline/halothane arrhythmias

Cats were anaesthetized with chloralose (80 mg/kg) intravenously and then allowed to breathe a mixture of oxygen and 0.5% halothane. Arterial pressure was monitored with an inductive type pressure transducer connected to the left femoral artery and heart rate with a cardiotachometer. The electrocardiogram (Lead II) was recorded. The level of anaesthesia was adjusted from time to time to ensure that an adequate diastolic pressure (not less than 50 mm Hg) was maintained. Intravenous dosage of adrenaline was commenced at 1 μg/kg and increased, in geometric progression, until a ventricular arrhythmia developed. Some cats responded to 1 μg/kg but others needed as much as 16 μg/kg. The mean dose required for ten cats was 6 ± 1.5 μg/kg. Following the establishment of three control arrhythmias, either (−)-propranolol or (+)-propranolol was given intravenously commencing at 0.05 and 1.0 mg/kg respectively. The dose was increased until the arrhythmia to adrenaline was abolished. The original arrhythmic dose of adrenaline was then doubled and in all cases arrhythmias similar to the control patterns were observed.

Ouabain-induced arrhythmias

For these experiments, cats (1.5–2.5 kg) and beagle dogs (9–12 kg) of either sex were anaesthetized with pentobarbitone (30 mg/kg). Heart rate, Lead II electrocardiogram (e.c.g.) and blood pressure were monitored throughout. The right vagus nerve was exposed and prepared for electrical stimulation (4–10 V ; duration 1 msec; frequency 20/sec) distally, following section in the mid-cervical region. The voltage was adjusted to produce a marked sinus bradycardia without cardiac arrest. Ventricular tachycardia was induced by intravenous ouabain (40 μg/kg), supplemented by a second dose of 20 μg/kg 30 min later. Additional doses of 10 μg/kg were given at 15-min intervals until ventricular arrhythmias were established. The absence of any response to vagal stimulation was used to demonstrate the completeness of ventricular dominance. The arrhythmias were allowed to stabilize for 10 min and then either saline or the compound under test was administered by continuous intravenous infusion. Compounds were given to cats at a rate of 0.5 mg/min (about 0.25 mg/kg/min), and to dogs at 2 mg/min (about 0.2 mg/kg per min), doses being calculated as base. In some instances, noted in the results section, higher infusion rates were employed.

The criteria used to demonstrate antiarrhythmic activity were (*a*) the reversion to normal sinus rhythm for at least 30 min, and (*b*) the failure of vagal stimulation to expose ectopic ventricular beats.

In some experiments insulin (80 u.) was given intravenously following the 30-min period of sinus rhythm to show that the reversal was not due to elimination or detoxification of the ouabain (insulin restored the ventricular tachycardia).

Results have been expressed as means $\pm$ standard error for groups of five animals.

Drugs

The following drugs were used: (−)-adrenaline bitartrate (Burroughs Wellcome); (±)-isoprenaline sulphate (Burroughs Wellcome); (±)-propranolol hydrochloride

(I.C.I.). The (+) and (−) isomers of propranolol were separated by Dr. T. Leigh of the Chemical Research Department, I.C.I., as the hydrochlorides, and were at least 99% pure as shown by measurement of optical rotation. Drugs were dissolved in 0.9% saline at the required concentration: all doses have been expressed in terms of the base.

Results

Blockade of cardiac responses to isoprenaline (anaesthetized rats)

At a dose level of 10 mg/kg subcutaneously, both isomers of propranolol and the racemic mixture produced a highly significant bradycardia. Whereas pretreatment with (−)-propranolol or racemic propranolol completely prevented an isoprenaline (0.1 μg/kg, intravenously) tachycardia, the response was not modified by (+)-propranolol. The results are summarized in Table 1.

In further experiments when the doses were reduced, a 50% block of the tachycardia produced by isoprenaline (0.1 μg/kg) was obtained with the racemate (200 μg/kg) or the (−) isomer (100 μg/kg). As (+)-propranolol (10 mg/kg) had no antagonistic activity, the potency of the (+) isomer is probably much less than 1/100th of that of the (−) isomer in this preparation.

Blockade of cardiac responses to isoprenaline (anaesthetized dogs)

The isomers of propranolol have also been compared for their ability to antagonize isoprenaline-induced increments in heart rate and cardiac contractile force in anaesthetized dogs. In each animal geometrically increasing doses of isoprenaline were given intravenously until a maximum response was obtained. Following the administration of the lowest dose of antagonist, the response curve to isoprenaline was reconstructed and the upper scale of dosage extended until a response in excess of 70% the maximum was obtained. This procedure was repeated for two higher dose levels of antagonist and a typical result for (−)-propranolol on the heart rate is illustrated in Fig. 1.

The dose-response curve is shifted to the right in parallel fashion as would be expected for a competitive antagonist. Similar curves were obtained for (±)-propranolol but for (+)-propranolol only at the 4.0 mg/kg dose level was there a significant displacement. The graphs for the change in heart rate remained parallel and it was possible to produce a maximum response equal to the control maximum. For changes in cardiac contractile force, there was a progressive decrease in the maximum response attainable.

Analysis of the results showed that the (−) isomer of propranolol was approximately twice as potent as the racemate. Significant antagonism with the (+) isomer,

TABLE 1. *Effect of racemic and isomeric forms of propranolol (10 mg/kg subcutaneously) on the resting heart rate of rats anaesthetized with pentobarbitone and on the response to isoprenaline (0·1 μg/kg)*

Treatment	Saline	(±)-propranolol	(+)-propranolol	(−)-propranolol
Control	383±12	379±13	387±16	368±9
30 min after drug	386±9	329±12	341±8	311±10
Change	+3	−50	−46	−57
Isoprenaline	486±9	327±8	444±12	312±6
Change	+100	−2	+103	+1

Each value represents the mean with standard error for twelve rats.

however, was only observed at the highest dose level tested (4 mg/kg). Qualitatively, the effects of the isomers were different. Whereas (−)-propranolol (0.25 mg/kg) produced an 18-fold block without there being any significant change in the *PR* interval of the electrocardiogram, (+)-propranolol (4.0 mg/kg) produced only a three-fold block but also a 30% increase in *PR* interval ($P<0.05$).

Blockade of metabolic responses to isoprenaline (anaesthetized dogs)

In addition to cardiovascular responses, the intravenous infusion of isoprenaline produces an increase in the circulating levels of glucose, free fatty acids and lactate. When infusions of isoprenaline (0.5 μg/kg/min) are repeated, the size of the response is smaller (Table 2). The decreases in response were not statistically significant, even for the changes in free fatty acids and heart rate where they were most marked. Following the infusion of (−)-propranolol (5 μg/kg/min), there was a virtually complete blockade of all three metabolic responses (Fig. 2). In contrast, (+)-propranolol at either 40 or 100 μg/kg/min was without effect on the fatty acid mobilization responses. Surprisingly, however, there was a reduction in both glucose and lactate responses which appeared to be dose dependent. At the higher dose level of (+)-propranolol there was also a statistically significant reduction in the heart rate response to isoprenaline. The total dose of (+)-propranolol infused by the end of the isoprenaline infusion was 2.5 mg/kg, so it was possible that some depression of conduction had occurred. The control tachycardia in this group was higher than in the others even though the final response was similar to that in the groups treated with saline or low doses of (+)-propranolol.

Negative inotropic activity (isolated guinea-pig atria)

Both isomers of propranolol depressed the force of contraction of electrically driven left atria taken from the hearts of guinea-pigs. For (+)-propranolol the mean concentration required to produce a 50% depression of control contractile force was $3.5 \pm 0.6 \times 10^{-4}$M (mean ± standard error), compared with $4.5 \pm 0.6 \times 10^{-4}$M for (−)-propranolol. The difference between these values was not statistically significant (five atria were used for each isomer).

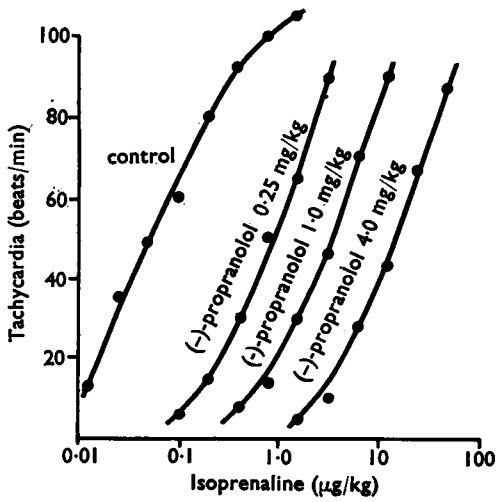

FIG. 1. Increments in heart rate to ascending doses of isoprenaline in an anaesthetized dog before and after various doses of (−)-propranolol. Control heart rate, 170 beats/min.

Local anaesthesia (isolated frog nerve)

There seemed to be no difference in the local anaesthetic potency of (+)- and (−)-propranolol as judged by their effect on conduction in an isolated nerve trunk. The mean concentration (with standard error; $n=5$) of (−)-propranolol required to produce a 50% reduction in spike amplitude was 20.2 ± 0.5 μg/ml., compared with 20.6 ± 1.6 μg/ml. for (+)-propranolol (Fig. 3). In a number of experiments it was found that direct comparison of (±)-propranolol with either isomer always showed a greater potency for the single isomer. A mixture of equal parts of pure (+)- and (−)-propranolol was prepared and tested under the same conditions as the isomers. The mean 50% blocking concentration was found to be 28.5 ± 1.3 μg/ml. This value was significantly different ($P<0.01$) when compared with the value for either isomer. For comparison, the concentration of procaine required to produce a 50% reduction in spike amplitude was 61 ± 4.5 μg/ml.

Antagonism of adrenaline/halothane arrhythmias (anaesthetized cats)

Intravenous injections of adrenaline produced a marked rise in blood pressure and multiple ventricular extrasystoles. The average rise in systolic blood pressure associated with arrhythmias was 135 mm Hg, representing an absolute value of 233 mm Hg. In individual cats, however, there was a wide variation in the final systolic pressure at which arrhythmias occurred ranging from 145 to 280 mm Hg. The dose of adrenaline required to produce arrhythmias also varied considerably, ranging

TABLE 2. *Effect of isoprenaline infusion (0·5 μg/kg/min) on metabolic responses before and after intravenous infusion of saline, (−)-propranolol or (+)-propranolol in anaesthetized dogs*

	Control	Isoprenaline	Change		Control	Isoprenaline	Change	% Block
				Saline				
FFA (μ-equiv/l.)	537±50	1,664±450	+1,127	↓	633±75	1,497±200	+864	23
Glucose (mg/100 ml.)	77·1±4·3	116·2±4·0	+39·1		76·7±1·1	112·3±12·2	+35·6	9
Lactate (mg/100 ml.)	9·2±1·1	18·4±1·1	+9·2		9·8±0·7	18·2±0·6	+8·4	8
Heart rate (beats/min)	153±7	216±11	+63		136±8	182±17	+46	27
				(−)-propranolol (5 μg/kg/min)				
FFA (μ-equiv/l.)	485±22	1,877±548	+1,392	↓	591±92	730±100	+139	90*
Glucose (mg/100 ml.)	66·9±7·0	128·9±9·1	+62·0		68·9±1·2	67·8±2·7	+3·9	94*
Lactate (mg/100 ml.)	5·5±0·9	14·3±0·6	+8·8		11·7±5·0	11·7±2·7	0	100*
Heart rate (beats/min)	159±8	238±15	+79		126±17	130±16	+4	95*
				(+)-propranolol (40 μg/kg/min)				
FFA (μ-equiv/l.)	663±89	1,543±446	+880	↓	675±147	1,584±400	+909	—
Glucose (mg/100 ml.)	85·0±2·7	152·2±31·7	+67·2		90·6±1·7	114·2±4·2	+23·6	65*
Lactate (mg/100 ml.)	11·4±3·8	17·5±7·1	+6·1		11·9±3·2	13·8±5·4	+1·9	69*
Heart rate (beats/min)	185±5	235±5	+50		172±8	218±3	+46	8
				(+)-propranolol (100 μg/kg/min)				
FFA (μ-equiv/l.)	625±61	1,109±201	+484	↓	475±53	1,056±263	+581	—
Glucose (mg/100 ml.)	83·5±2·6	134·5±9·2	+51·0		91·6±3·0	100·7±9·1	+9·1	82*
Lactate (mg/100 ml.)	12·9±4·9	23·1±3·1	+10·2		13·6±3·3	15·7±3·7	+2·1	79*
Heart rate (beats/min)	175±15	278±3	+103		155±5	200±10	+45	56*

Each block represents the mean with standard errors for a group of three dogs. An asterisk indicates significant blockade ($P<0·05$).

from 1 to 16 μg/kg). The mean duration of arrhythmias was 46±7 sec. No clear correlation emerged between the pressor response, final systolic values or change in pulse pressure and the arrhythmic dose of adrenaline. Nor was there a correlation between the adrenaline dosage and the duration of arrhythmias. Both isomers of propranolol were able to prevent adrenaline arrhythmias in cats anaesthetized with halothane. The average dose (with S.E.) of (+)-propranolol required was 4.2±1.2 mg/kg, whereas that of (−)-propranolol was 0.09±0.02 mg/kg (five cats for each isomer). Some of the effects of these antiarrhythmic doses of propranolol isomers are summarized in Table 3. Effective doses of (+)-propranolol produced a significant lowering of the heart rate ($P<0.05$), had little action on the blood pressure but markedly increased the *PR* interval of the electrocardiogram ($P<0.001$). When the previously arrhythmic dose of adrenaline was repeated, there was a modest sinus tachycardia and a pressor response similar to that before (+)-propranolol. The antiarrhythmic dose of (−)-propranolol had no significant effect on heart rate, blood pressure or the *PR* interval. After the arrhythmic dose of adrenaline there was little evidence of a change in heart rate but the same pressor response was observed as in the control period. The final systolic pressure was lower but this observation was not statistically significant.

If the arrhythmic dose of adrenaline was doubled following the antiarrhythmic dose of either isomer of propranolol, arrhythmias of duration and intensity similar

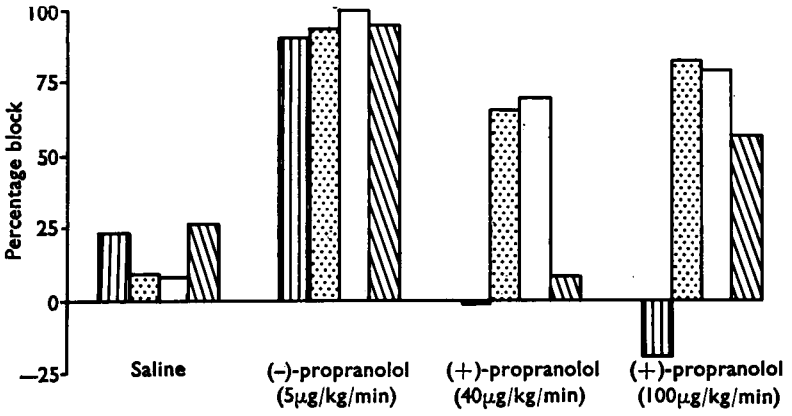

FIG. 2. Percentage reduction in the metabolic responses to isoprenaline (0.5 μg/kg/min) following the intravenous infusion of various doses of (−)- and (+)-propranolol in anaesthetized dogs. ▥ Free fatty acids; ▨, blood sugar; □ plasma lactate; ▧, heart rate.

TABLE 3. *Effect of (+) and (−)-propranolol on cardiovascular system of cats, anaesthetized with halothane, before and after an arrhythmic dose of adrenaline*

	Antiarrhythmic dose (mg/kg) (mean±S.E.)	Before adrenaline Heart rate (beats/min)	Blood pressure (mm Hg)	*PR* interval (msec)	After adrenaline Heart rate (beats/min)	Blood pressure (mm Hg)
Control period	—	139	107/71	86±1·1	191	254/181
After (+)-propranolol	4·2±1·2	122*	104/69	103+2·0*	137*	236/180
Control period	—	144	88/56	98±0·7	204	210/156
After (−)-propranolol	0·09±0·02	138	78/46	95±0·7	138*	187/146

The values are means from five cats for each isomer. An asterisk indicates a significant difference from the control figure ($P<0·05$).

to the control arrhythmias were obtained. From these results the ratio of effective doses of (+)- and (−)-propranolol were 47:1.

Antagonism of ouabain arrhythmias (anaesthetized cats)

The total dose of ouabain required to produce a stable ventricular tachycardia in cats anaesthetized with pentobarbitone ranged from 60 to 140 μg/kg. Of five animals infused with saline, two died from ventricular fibrillation after 40 and 60 min respectively, two maintained stable arrhythmias for longer than 3 hr and one showed a spontaneous return to sinus rhythm after 2 hr. Cats which received an intravenous infusion of (−)-propranolol at a fixed rate of 0.5 mg/min all showed a reversion to sinus rhythm within 8–15 min, the mean total dose being 1.90±0.29 mg/kg. The average heart rate after sinus rhythm had been restored was 60 beats/min lower than the original rate. If the infusion rate was increased to 2.0 mg/kg/min, two out of two cats died after 5 and 9 min of infusion respectively, without a return to sinus rhythm. In a third cat non-continuous infusion over 17 min led to restoration of normal rhythm 5 min later after a total dose of 8 mg/kg. Cats which received an intravenous infusion of (+)-propranolol at 0.5 mg/min also exhibited a prompt return to sinus rhythm after a mean total dose of 6.5±0.9 mg/kg. The final heart rate was an average of 48 beats/min below the initial rate. The difference between the antiarrhythmic doses of (+)- and (−)-propranolol was statistically significant ($P<0.01$). These results are summarized in Table 4.

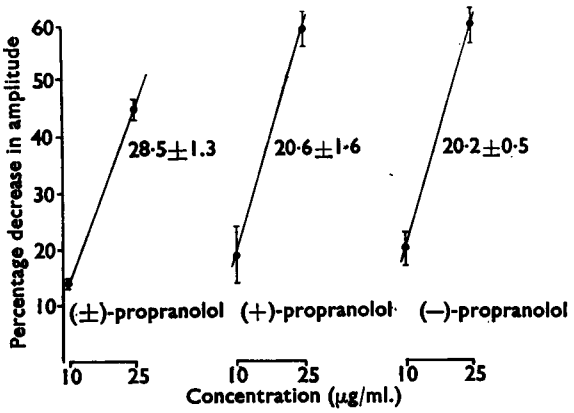

FIG. 3. Effect of (±)-, (+)- and (−)-propranolol on the spike amplitude in isolated frog sciatic nerves. The mean concentration producing 50% reduction is shown together with the standard error ($n=5$).

TABLE 4. *Reversal of ouabain induced arrhythmias by (+) and (−)-propranolol in anaesthetized cats*

No. of cats	Dose of ouabain (μg/kg)	Drug infused	Infusion rate (per min)	No. of reversals	Anti-arrhythmic dose (mg/kg)	Heart rate (beats/min) Before	After
5	104±12	Saline	0·15 ml.	1/5	—	190	187
4	77±8	(−)-propranolol	0·5 mg	4/4	1·90±0·29	171±21	111±9
3	100±10	(−)-propranolol	2·0 mg/kg	1/3	died		
					died		
					8·0		
5	94±15	(+)-propranolol	0·5 mg	5/5	6·48±0·9	182±8	134±11
3	86±3	(+)-propranolol	1·0 mg		8·65	200	100
			1·0 mg	2/3	died	—	—
			2·0 mg/kg		5·0	183	160

Mean values are given ±S.E.

Antagonism of ouabain arrhythmias (anaesthetized dogs)

It proved considerably easier to induce a sustained ventricular tachycardia in dogs, compared with cats. The dose of ouabain required ranged from 60 to 80 μg/kg. Animals infused with saline showed spontaneous reversal to sinus rhythm within 1.5–4 hr of onset of the arrhythmia. The infusion of (−)-propranolol produced a prompt reversal of the arrhythmia within 5–10 min of the beginning of infusion. The mean dose required was 2.5 ± 0.63 mg/kg. The total mean dose of (+)-propranolol was higher for complete reversal being 4.4 ± 0.29 mg/kg, the difference being statistically significant ($P<0.05$). In some experiments, sinus rhythm was restored at doses at low as 0.5–1.0 mg/kg but it was necessary to give further compound to ensure maintenance of normal rhythm for 30 min and to provide complete protection against ventricular ectopics during vagal stimulation. This tendency to require more compound for protection during vagal bradycardia was more marked with (+)-propranolol. A typical set of experiments is illustrated in Fig. 4 and the complete results summarized in Table 5.

The antiarrhythmic doses of either (+)- or (−)-propranolol had little effect on systemic blood pressure when the values before ouabain and after reversal to sinus

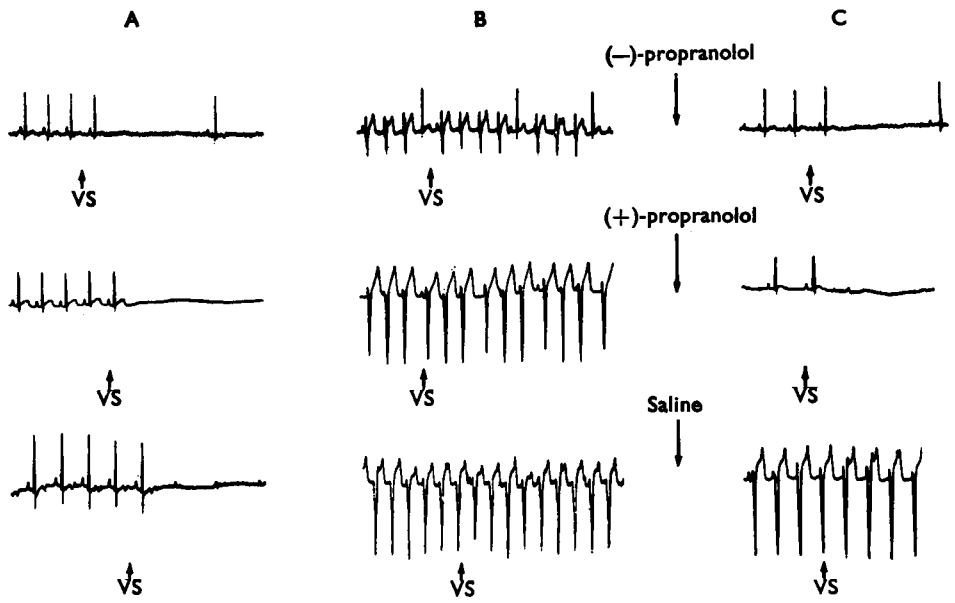

FIG. 4. Reversal of ouabain-induced arrhythmias by (+)- and (−)-propranolol in anaesthetized dogs as shown by Lead II e.c.g. recordings. V.S., Vagal stimulation; paper speed, 2.5 cm sec^{-1}. A, Control; B, ventricular tachycardia produced by ouabain; C, 30 min after infusion of propranolol isomer or 2 hr after infusion of saline.

TABLE 5. *Reversal of ouabain arrhythmias by (+)- and (−)-propranolol in anaesthetized dogs*

No. of dogs	Dose of ouabain (μg/kg)	Drug infused	Infusion rate (per min)	Anti-arrhythmic dose (mg/kg)	Heart rate (bears/min) Before	Heart rate (bears/min) After	Blood pressure (mm Hg) Before	Blood pressure (mm Hg) After
5	68±4	Saline	0·15 ml.	—	134±12	136±16	190/132	200/130
5	62±2	(−)-propranolol	2 mg	2·5±0·63	148±15	124±15	167/113	180/114
5	64±2	(+)-propranolol	2 mg	4·4±0·29	139±3	109±8	190/113	188/106

Mean values are given ±S.E.

rhythm were compared. No correlation was found between the dose of ouabain and the antiarrhythmic dose of propranolol isomer or the duration of arrhythmias.

Discussion

The results of this investigation afford conclusive evidence that the laevo isomer of propranolol is responsible for the β-receptor antagonist properties of the racemic compound. Whether or not the dextro isomer possesses any β-receptor antagonist potential is a question unlikely to be resolved satisfactorily because its effects on conduction will inevitably tend to diminish the effects of β-receptor stimulation as happens with iproveratril (Fitzgerald & Barrett, 1967). From experiments in rats, both isomers were found to have similar negative chronotropic activity at high dose levels, although (+)-propranolol had no effect on the chronotropic response to isoprenaline. In cats and dogs higher doses of (+)-propranolol did produce some alteration of the cardiac responses to isoprenaline, but only at levels which also produce a significant prolongation of the *PR* interval of the electrocardiogram.

In dogs which had been fasted for 20 hr the mobilization of free fatty acids in response to isoprenaline was completely suppressed by doses of (−)-propranolol while up to 20 times the amount of the (+) isomer was without effect. Suppression of the fatty acid response has previously been reported for racemic propranolol in rats (Salvador, April & Lemberger, 1967), dogs (Zsoter, Tom, Kraml & Dvornik, 1966), and man (Antonis, Clark, Hodge, Molony & Pilkington, 1967). Similar results for the two isomers of 1-(*o*-allylphenoxy)-3-isopropylamino-2-propranol (H 56/28) on the mobilization of free fatty acids produced by noradrenaline have also been observed (Björntorp, Ek, Olsson & Schröder, 1967). Earlier results also suggested that (−)-propranolol would effectively prevent the changes in blood glucose and lactate as shown in the present study. In contrast, the finding that (+)-propranolol exerted a significant inhibitory action on both glucose and lactate responses was most unexpected. Considerable confusion exists in the literature as to the type of receptor involved in the changes in glucose and lactate brought about by catecholamines. In addition to direct effects, it is possible that some changes may be a secondary response to cardiovascular changes (Grayson & Johnson, 1953). Alternatively the present results may be considered to support the views of Lundholm & Mohme-Lundholm (1960), who believe that catecholamine-induced vasodilatation is a secondary consequence of the stimulation of carbohydrate metabolism in muscle and the accumulation of products such as lactic acid. Shanks (1967) has shown (+)-propranolol to have one-twentieth the potency of (+)-propranolol in preventing isoprenaline induced changes in hind limb blood flow and this isomer has also been observed to convert the depressor response of isoprenaline to a pressor response (Whitsitt & Lucchesi, 1967). Butoxamine, described as an atypical β-receptor antagonist, in that it prevents the metabolic but not the cardiac effects of isoprenaline, has been shown to antagonize isoprenaline induced vasodilatation (Levy, 1966). Preliminary experiments with 4-(2-hydroxy-3-isopropyl-aminopropoxy) acetanilide (I.C.I. 50,172), which antagonizes β-receptors in the heart and inhibits fatty acid mobilization but not peripheral β-receptors in the circulation (Barrett, Crowther, Dunlop, Shanks & Smith, 1968), show that it

possesses much less inhibitory action on lactate and glucose responses than does propranolol.

That both isomers of propranolol should exhibit similar negative inotropic activity was anticipated since Parmley & Braunwald (1967) found no difference between the dextro isomer and the racemic compound in this respect. It was, therefore, surprising to find a significant difference between the local anaesthetic potencies of the isomers and racemic propranolol because many workers have agreed that there is a close correlation between negative inotropic and local anaesthetic activity. In the present study, the technique employed would not permit the detection of a two-fold difference with the atrial preparation, but this criticism could not be applied to the work of Parmley & Braunwald (1967).

Chemical evidence suggests that the isomers of propranolol combine to form a racemic compound and there may well be, therefore, differences in molecular volume accounting for the differences in potency. Another investigation has shown that MJ 1999, at concentrations having no effect on spike amplitude, will significantly reduce the depressant effects of procaine on isolated frog nerves (Åberg & Welin, 1967). Similarly, a mixture of racemic propranolol and procaine was shown to be less active than either applied separately. It was suggested that a β-receptor antagonist might delay the penetration of nerves by local anaesthetics but the present results do not support such a conclusion.

Both isomers of propranolol are completely effective in reversing experimental arrhythmias but at different dose levels. In the case of arrhythmias produced by adrenaline under halothane anaesthesia, the results suggest that (−)-propranolol may act by competitively preventing the access of adrenaline to cardiac β-receptors, whereas (+)-propranolol most probably acts at a more distal site by preventing the increased sinus rate being fully transmitted to the ventricles. Support for this view comes from the electrocardiogram findings.

The comparison of the activity of the isomers of propranolol in reversing ouabain-induced arrhythmias proved particularly interesting. In both cats and dogs, the (−) isomer was consistently more effective than (+)-propranolol in quantitative terms and this difference was statistically significant. These findings differ from previous reports in the literature in two important ways. First, in the only other comparison of the (+) and (−) forms in this context, Howe and Shanks (1966) concluded that (−)-propranolol did not affect the process of ventricular tachycardia induced by ouabain, whereas (+)-propranolol was effective. It was considered that the results indicated that reversal of ouabain arrhythmias was unrelated to either β-blockade or local anaesthetic activity. In our hands the infusion of (−)-propranolol at a rate of 2 mg/kg/min, the dose level adopted by Howe & Shanks (1966), consistently produced signs of overt toxicity. Infusion at one tenth this level has produced complete reversal of arrhythmias without signs of toxicity. It is most probable that this difference in dose accounts for the discrepancy between the two investigations. Second, the greater activity of (−)-propranolol than the (+) isomer against ouabain-induced arrhythmias suggests that ability to block adrenoceptive β-receptors may, after all, be associated with this property provided that the compound also has local anaesthetic properties.

Lucchesi, Whitsitt & Brown (1966) and Whitsitt & Lucchesi (1967) concluded that β-blockade was of no consequence in the reversal of ouabain arrhythmias because

the dose levels of propranolol required to reverse arrhythmias were considerably greater than those for β-blockade and that (+)-propranolol was effective at dose levels not exhibiting β-blockade. Further, Sekiya & Vaughan Williams (1963) clearly demonstrated the similarities of action between pronethalol, procaine and quinidine in reversing ouabain toxicity, and related the effects to local anaesthetic properties. Neither N-isopropyl-*p*-nitrophenylethanolamine (Tuttle & Innes, 1964), nor I.C.I. 50,172 (Dunlop & Shanks, 1968), which lack local anaesthetic activity, reversed ouabain arrhythmias. From these results it appears that β-blocking activity alone will not reverse ouabain arrhythmias but once an effect on conduction has been established, then β-blockade would seem to potentiate the effect and reduce the total dosage required. It seems, therefore, that local anaesthetic properties are essential for antiarrhythmic activity whereas adrenergic β-receptor blocking activity is not. Our findings with the isomers of propranolol, however, indicate that it may be an advantage in compounds which already possess local anaesthetic properties.

REFERENCES

ÅBERG, G. & WELIN, I. (1967). Interaction of beta-receptor blocking agents with the nerve blocking effects of procaine in vitro. *Life Sci.*, **6**, 975–979.

ANTONIS, A., CLARK, M. L., HODGE, T. L., MOLONY, M. & PILKINGTON, T. R. E. (1967). Receptor mechanisms in the hyperglycaemic response to adrenaline in man. *Lancet*, **1**, 1135–1137.

ARIËNS, E. J. (1967). The structure-activity relationships of beta adrenergic drugs and beta adrenergic blocking drugs. *Ann. N.Y. Acad. Sci.*, **139**, 606–631.

BARRETT, A. M., CROWTHER, A. F., DUNLOP, D., SHANKS, R. G. & SMITH, L. H. (1968). Cardioselective β-Blockade. *Arch. exp. Path. Pharmak.*, **259**, S. 152.

BARRETT, A. M. & THORP, J. M. (1968). Studies on the mode of action of clofibrate: effects on hormone-induced changes in plasma free fatty acids, cholesterol, phospholipids and total esterified fatty acids in rats and dogs. *Br. J. Pharmac. Chemother.*, **32**, 381–391.

BJÖRNTORP, P., EK, L., OLSSON, S. & SCHRÖDER, G. (1967). The effect of a β-adrenergic receptor antagonist (H 56/28) on lipid mobilization. *Acta pharmac. tox.*, **25**, suppl. 2, 51–62.

BLINKS, J. R. (1965). Convenient apparatus for recording contractions of isolated heart muscle. *J. appl. Physiol.*, **20**, 755–757.

DUNLOP, D. & SHANKS, R. G. (1968). Selective blockade of adrenoceptive beta receptors in the heart. *Br. J. Pharmac. Chemother.*, **32**, 201–218.

FITZGERALD, J. D. & BARRETT, A. M. (1967). What is a β-blocker? *Lancet*, **2**, 310.

GILL, E. W. & VAUGHAN WILLIAMS, E. M. (1964). Local anaesthetic activity of the β-receptor antagonist, pronethalol. *Nature, Lond.*, **201**, 199.

GRAYSON, J. & JOHNSON, D. H. (1953). The effect of adrenaline and noradrenaline on the liver blood flow. *J. Physiol., Lond.*, **120**, 73–94.

HORSFALL, G. B. (1965). A wide-range, high discrimination cardiotachometer. *J. Physiol., Lond.*, **180**, 1*P*.

HOWE, R. (1963). Structure activity relationships of some beta-adrenergic blocking agents. *Biochem. Pharmac.*, **12**, suppl., 85–86

HOWE, R. & SHANKS, R. G. (1966). Optical isomers of propranolol. *Nature, Lond.*, **210**, 1336–1338.

LEVY, B. (1966). Adrenergic blocking activity of N-tertiary butymethoxamine (butoxamine). *J. Pharmac. exp. Ther.*, **133**, 202–210.

LISH, P. M., WEIKEL, J. H. & DUNGAN, K. W. (1965). Pharmacological and toxicological properties of two new beta-adrenergic receptor antagonists. *J. Pharmac. exp. Ther.*, **149**, 161–173.

LUCCHESI, B. R. (1964). The action of nethalide upon experimentally induced cardiac arrhythmias. *J. Pharmac. exp. Ther.*, **145**, 286–291.

LUCCHESI, B. R. (1965). The effects of pronethalol and its dextro isomer upon experimental cardiac arrhythmias. *J. Pharmac. exp. Ther.*, **148**, 94–99.

LUCCHE I, B. R., WHITSITT, L. S. & BROWN, N. L. (1966). Propranolol (Inderal) in experimentally-induced cardiac arrhythmias. *Can. J. Physiol. Pharmac.*, **44**, 543–547.

LUNDHOLM, L. & MOHME-LUNDHOLM, E. (1960). The action of adrenaline on carbohydrate metabolism in relation to some of its pharmacodynamic effects. In *Adrenergic Mechanisms*, ed. Vane, J. R., pp. 305–322. London: Churchill.

PARMLEY, W. W. & BRAUNWALD, E. (1967). Comparative myocardial depressant and anti-arrhythmic properties of d-propranolol, *dl*-propranolol and quinidine. *J. Pharmac. exp. Ther.*, **158**, 11–21.

SALVADOR, R. A., APRIL, S. A. & LEMBERGER, L. (1967). Inhibition by butoxamine, propranolol and MJ 1999 of the glycogenolytic action of the catecholamines in the rat. *Biochem. Pharmac.*, **16**, 2037–2041.

SEKIYA, A. & VAUGHAN WILLIAMS, E. M. (1963). A comparison of the anti-fibrillatory actions and effects on intracellular cardiac potentials of pronethalol, disopyramide and quinidine. *Br. J. Pharmac. Chemother.*, **21**, 473–481.

SHANKS, R. G. (1967). The peripheral vascular effects of propranolol and related compounds. *Br. J. Pharmac. Chemother.*, **29**, 204–217.

TUTTLE, R. R. & INNES, I. R. (1964). Antagonism of ouabain-induced arrhythmias by nethalide. *Pharmacologist*, **6**, 165.

WHITSITT, L. S. & LUCCHESI, B. R. (1967). The cardiac beta-adrenergic receptor blocking actions of propranolol and its stereo-isomers. *Life Sci.*, **6**, 939–950.

ZSOTER, T., TOM, H., KRAML, M. & DVORNIK, D. (1966). Adrenergic beta receptors and plasma free fatty acids. *J. Pharmac. exp. Ther.*, **152**, 425–431.

(*Received April* 8, 1968)