Kamer (1955) showed in 50 children with coeliac disease that there was a greater rise in blood glutamine after oral gliadin than in control subjects. Similar observations have been made after feeding gluten to adult coeliac patients. Douglas and Booth (1969) studied nine control subjects and six patients with coeliac disease before and after treatment with a gluten-free diet. The amino-acids in the blood were measured by ion exchange chromatography before and after giving an oral load of 25 g. of gluten (Fig. 14). In the untreated coeliac patients there was a surprising and striking increase in the amino-acids above normal levels at two hours, but the pattern became normal after treatment with a gluten-free diet. This observation is difficult to explain, but it is possible that in untreated coeliac patients in whom there are secondary deficiencies of enzymes concerned with protein digestion peptides and polypeptides may escape digestion during absorption and pass on into the body relatively unchanged. They may then only be more slowly broken down to amino-acids by the liver, thus yielding high levels in the untreated state. The normal results after treatment may be particularly significant, since they suggest that the mucosa is functionally normal so far as gluten is concerned after treatment. They provide no support for the hypothesis of a specific enzyme defect.

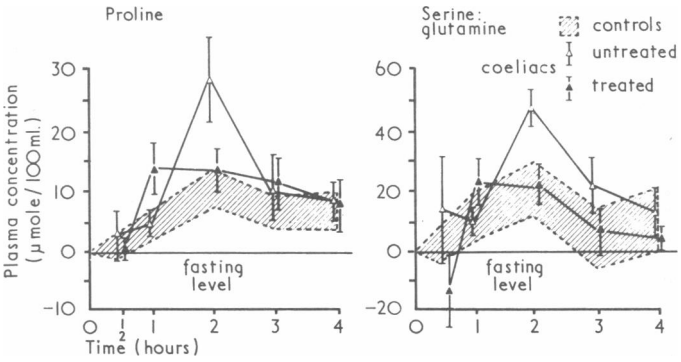

FIG. 14.—Plasma amino-acid curve after feeding 25 g. of gluten to control subjects and patients with coeliac disease before and after treatment. (From Douglas and Booth, 1969.)

The conclusion of this lecture, with a list of references, will be published in our next issue.

Plasma Propranolol Levels in the Quantitative Assessment of ß-adrenergic Blockade in Man

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British Medical Journal, 1970, 3, 731-734

Summary: Plasma propranolol levels associated with reductions in endogenous and exogenous cardiac β-stimulation were determined in normal people. The levels associated with a given degree of blockade of exercise-induced tachycardia were about three times greater after intravenous administration than after oral administration. This shows that an active metabolite of propranolol is formed only after the drug is taken by mouth. The greatest reduction in the tachycardia of strenuous exercise was associated with plasma levels of 40 ng./ml. with oral administration and 100 ng./ml. with intravenously administered propranolol.

The effect on isoprenaline-induced tachycardia following intravenously administered propranolol showed that the dose ratio for isoprenaline was about 30 with plasma levels of 100 ng./ml. and 10 with levels of 10-20 ng./ml. These plasma levels give 100% and 20-30% blockade of exercise-induced tachycardia. These findings suggest that some of the therapeutic effects of propranolol may be unrelated to β-adrenergic blockade.

Introduction

There is surprisingly little information about the quantitative aspects of β-adrenergic blockade in man. For example, it is not yet possible to predict the dose of drug required to produce a given degree of β-adrenergic blockade in a given patient. Furthermore, the dose used may be greater than that required for complete blockade of endogenous sympathetic stimulation, when another property of the drug might be responsible for its therapeutic effect. In pharmacological terms an accurate knowledge of the dose/response relationship is required, but the precise measurement of both dose and response has proved difficult in practice.

The dose should be related to the concentration of the antagonist drug to which the target organ is exposed. In practice this requires a method for measuring the plasma levels of the drug, as the concentration of a drug in the plasma (and therefore reaching the target organ) after a fixed dose may vary widely among individuals, especially when the drug is given by mouth. A suitable test of β-adrenergic stimulation has also posed problems, for it should be capable of producing with safety a maximal effect which, if possible, involves an increase in sympathetic stimulation rather than withdrawal of vagal tone. All the simple tests available have some disadvantages, but the tachycardia produced by exercise appears to be the most satisfactory, for if the work is strenuous it does not seem to be mediated by change in vagal tone (Robinson *et al.*, 1953).

An exogenous stimulus, such as isoprenaline, can be used, but its effects have not yet been related to those of endogenous sympathetic stimulation, and it does not follow that, if the effects of a given dose of isoprenaline are abolished by an antagonist, those of sympathetic nerve stimulation will be affected to the same extent. The test procedure having been defined, a full range of doses of the antagonist, including those producing a maximal effect, should be investigated.

The present study was designed to define the plasma-concentration/dose-response relationship for propranolol by measuring the degree of blockade of the tachycardia produced by exercise at various work loads. The antagonism of an isoprenaline-induced tachycardia was then determined to compare the effects of the drug on endogenous and exogenous stimulation of cardiac β-receptors.

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Methods

Normal volunteer men aged 25-38 were studied. All were non-smokers, and the investigations were begun between 9 and 10 a.m. in the fasting state.

Exercise-induced Tachycardia

Six subjects each completed three treatments—placebo and 40 mg. and 80 mg. of propranolol in a randomized balanced double-blind study. Having taken the tablets one and a half hours before study, the subjects rested supine for 30 minutes and the heart rate was measured from a precordial E.C.G. by noting the duration of 10 R-R intervals. A plasma sample was then obtained and the subjects exercised for two-and-a-half minutes on a bicycle ergometer, beginning with a work load of 50 watts and increasing in increments of 50 until the subject considered he was almost maximally fatigued. The heart rate was measured during the last 20 seconds of each exercise period. In three subjects there was no difference between the effects of 40 and 80 mg. of propranolol. In the other three the 40-mg. dose produced a smaller reduction in exercise tachycardia than did 80 mg. To determine the dose producing maximal effect the subjects underwent investigation of a third dose of the drug—20 mg. in the case of those who showed no difference in the effects of 40 and 80 mg., and 120 mg. in those subjects in whom 80 mg. had a greater effect than 40 mg. Thus for each subject a three-point dose/response curve was obtained, such that the lowest dose was submaximal and the middle and highest doses were maximal and produced identical effects.

The effects of an intravenous injection of saline or 0·3 mg. of propranolol per kg. over two minutes were compared in the same six subjects in a randomized balanced study. This dose was chosen after a pilot study had shown that it produced maximal blockade 15 minutes after the injection. The subjects were exercised at the greatest work load which, in the previous study, they could tolerate for at least two minutes after having taken the highest dose of propranolol. The heart rate was measured before and during exercise periods 15, 60, and 240 minutes after the injection. Plasma samples were obtained before the period of exercise.

Isoprenaline-induced Tachycardia

The tachycardia after intravenous injections as a bolus of increasing amounts of isoprenaline was determined in three subjects at rest and 15 and 240 minutes after 0·3 mg. of propranolol per kg. intravenously. In three other subjects the dose of propranolol was reduced to 0·15 mg./kg. Heart rate was recorded from the precordial E.C.G. and plasma samples were obtained before and after the isoprenaline tests. The heart rate was allowed to return to control levels between each dose of isoprenaline. Log dose/response plots to isoprenaline were made for the control and post-propranolol periods, and the ratio of the dose giving an increase of 25 beats/minute over the preinjection heart rate in the control period to those after propranolol was calculated. Plasma propranolol concentrations were measured fluorometrically as previously described (Shand *et al.*, 1970).

Results

Effects on Exercise-induced Tachycardia

The tachycardia resulting from various exercise loads after the oral administration of placebo and three doses of propranolol is shown in Table I. The effects of propranolol on the tachycardia during the most strenuous exercise are shown in Fig. 1. In five of the six subjects the effect of the middle dose was indeed maximal, for increase in dose (resulting in higher plasma levels) produced no further reduction in the tachycardia. In the sixth subject the highest dose produced a greater effect than the middle dose and was considered maximal. The lowest dose in each subject produced an effect less than maximal. The effects of 0·3 mg. of propranolol per kg. intravenously in the same subjects are shown in Table II. The effects at one hour and four hours were less than at 15 minutes and were therefore submaximal. At 15 minutes the effects were considered maximal because the heart rate attained was similar to that after the maximal effect of oral doses in each subject.

TABLE I.—*Effect of 3 Oral Doses of Propranolol on Tachycardia Induced by Graded Exercise in 6 Subjects*

Treatment	Heart Rate, Mean ± S.E.				
	Rest	Exercise Grade*			
		1	2	3	4
Placebo ..	62 ± 4·3	109 ± 3·1	132 ± 4·4	158 ± 4·9	179 ± 4·5
Low dose (L) ..	53 ± 4·2	96 ± 2·3	112 ± 3·1	132 ± 1·7	149 ± 2·5
Middle dose (M)	52 ± 3·9	91 ± 3·2	105 ± 2·0	118 ± 1·7	130 ± 1·4
High dose (H) ..	53 ± 3·6	94 ± 3·7	106 ± 2·2	119 ± 1·9	128 ± 1·5
t test diff. (L) from (M) or (H)	N.S.	N.S.	P<0·05	P<0·001	P<0·001

*Grade 4 work load was the maximum tolerated, being 300 watts for 3 subjects, 250 watts for 2, and 200 watts for 1 subject, and the exercise grades increase in 50 watt increments up to the maximum.

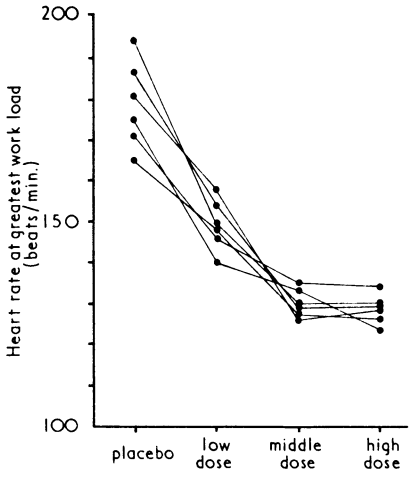

FIG. 1.—Effects of propranolol on the tachycardia induced by the most strenuous exercise.

TABLE II.—*Effect of 0·3 mg. of Propranolol per kg. Intravenously on Tachycardia Induced by Strenuous Exercise in 6 Subjects*

Treatment	Heart Rate, Mean ± S.E.					
	15 Min. after Inj.		60 Min. after Inj.		240 Min. after Inj.	
	Rest	Exercise*	Rest	Exercise*	Rest	Exercise*
Placebo	70 ± 4·9	171 ± 3·7	74 ± 4·3	173 ± 3·9	77 ± 6·0	172 ± 3·8
Propranolol ..	55 ± 6·0	122 ± 2·9	64 ± 3·3	134·5 ± 3·8	61 ± 3·9	146 ± 4·4

*Exercise was performed at the greatest exercise load tolerated in the study shown in Table I.

Plasma concentration/response curves were constructed for orally and intravenously administered propranolol (Fig. 2). The difference in heart rate at the greatest work load for placebo treatment and that after maximal blockade was taken as 100% and the effect of the submaximal dose calculated as a percentage of this difference. This has been plotted against the log of the plasma level present just before the exercise, and the lines have been fitted for the submaximal effects by the method of least squares. For a given effect about three times greater concentrations of propranolol were found after intravenous than after oral dosage. Notably there was no

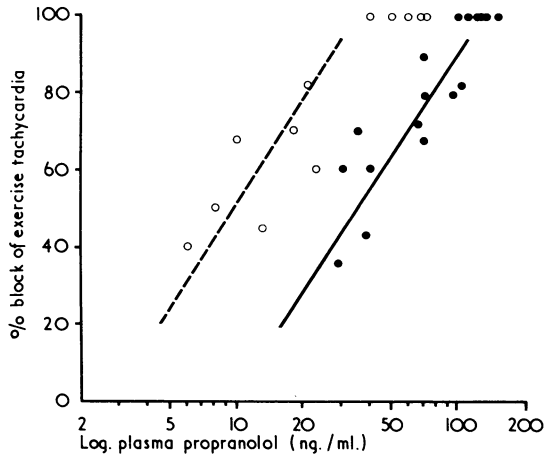

FIG. 2.—Log plasma concentration/response relationship for orally administered (O) and intravenously administered (●) propranolol.

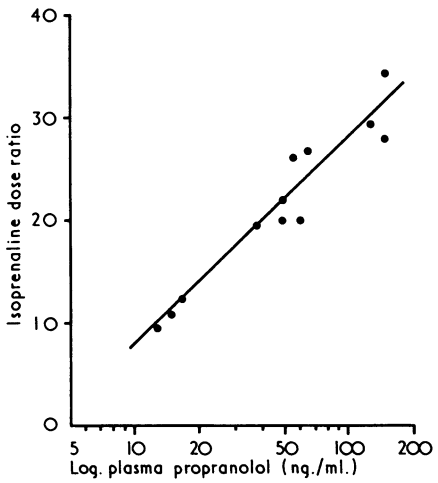

FIG. 3.—Relationship between the dose ratio for isoprenaline and the log plasma propranolol concentration.

overlap between the plasma levels associated with submaximal and maximal effects of the drug. Maximal blockade of exercise tachycardia is associated with plasma levels of greater than 40 ng./ml. after oral administration, and greater than 100 ng./ml. after intravenous administration.

Effects on Isoprenaline-induced Tachycardia

The log dose/response curves to isoprenaline after propranolol were parallel to those obtained in the control period, implying that the antagonism was competitive. The dose ratios have been calculated and are given in Table III.

TABLE III.—*Dose Ratios to Isoprenaline After Intravenous Propranolol*

Subject (Dose of Propranolol mg./kg.)	Control	15 Min. after Propranolol		240 Min. after Propranolol	
	Isop.$_{25}$μg.*	Isop.$_{25}$μg.*	Dose Ratio	Isop.$_{25}$μg.*	Dose Ratio
1 (0·30) ..	3·2	94·0	29·4	62·0	19·4
2 (0·30) ..	1·5	42·0	28·0	30·0	20·0
3 (0·30) ..	1·5	52·0	34·6	30·0	20·0
4 (0·15) ..	2·2	48·0	21·8	27·0	12·3
5 (0·15) ..	1·5	40·0	26·6	16·0	10·7
6 (0·15) ..	1·8	47·0	27·0	17·0	9·5

*Dose of isoprenaline, found by interpolations, of the log dose/response curve, which gives an increase in heart rate of 25 beats/minute over the resting value. The dose ratio is calculated as the ratio of the Isop.$_{25}$ after propranolol to that during the control period.

These dose ratios plotted against the log of the mean plasma propranolol concentrations before and after each isoprenaline test are shown in Fig. 3. A good straight-line relationship was obtained. A dose ratio of about 30 was found with levels of 100-150 ng./ml. and about 10 with levels of 10-20 ng./ml. From Fig. 2 these plasma levels would be associated with 100% and about 20% block of exercise tachycardia after intravenous administration.

Discussion

When attempting to quantify the effects of β-adrenergic blockade on the heart it is important to eliminate changes in vagal tone that might occur. In the present study the effects on the resting heart rate could not discriminate between submaximal and maximal doses of propranolol. This is not surprising, as it is well recognized that changes in resting heart rate depend as much on a change in vagal as in sympathetic tone (Chamberlain *et al.*, 1967). The same might be said of the tachycardia produced by tilt, Valsalva's manoeuvre, and mild exercise. Atropine can be used to abolish vagal effects, but the high doses required are unpleasant to take and therefore unsuitable for repeated studies in the same person. While the physiological response to exercise is complex, the withdrawal of vagal influence does not affect the associated tachycardia, provided that the exercise is strenuous enough to increase the heart rate in the placebo period to above about 130 beats/minute (Robinson *et al.*, 1966) or to more than 110 beats/minute after maximal β-blockade (Chamberlain *et al.*, 1967). This is an agreement with the present finding that submaximal and maximal effects could be consistently distinguished only when the heart rate during the placebo study had been greater than 130 beats/minute. because the change in resting heart rate is so influenced by vagal tone it has not been used in the calculation of the degree of blockade. Rather, 100% block has been defined as the difference between the tachycardia obtained at the greatest work load during the control period and that after maximally effective doses of propranolol at the same work load. The effect of a submaximally effective dose is then easily calculated as a percentage of the greatest effect obtainable.

Isoprenaline-induced tachycardia has also been used as a test procedure and is most accurate when a dose/response curve is established before and after the β-adrenergic blockade drug (Dollery *et al.*, 1969). Provided certain precautions are taken and specified limits set, the procedure is safe. For example, for each isoprenaline series it is important to begin with a small dose (0·25 μg. during the control period). This can be increased until an effect is produced. In practice we have found that doses of 0·5-4 μg. were required to establish the dose/response relationship in the control period. We consider that an increase in rate of 40 beats/minute or an absolute tachycardia of 110 beats/minute was a satisfactory and safe end-point for the test, and no ectopic beats were observed in the 18 tests performed. With a competitive antagonist like propranolol there is a parallel shift to the right of the dose/response curve for isoprenaline, and the antagonism can be measured by calculating the dose ratio at a given tachycardia. The greater the degree of antagonism the greater is this dose ratio, and because there is no limit to the amount of exogenous isoprenaline that can be given, this becomes a continuous variable within the limits of toxicity of the antagonist. The dose ratios obtained should therefore be related to the degree of blockade of endogenous sympathetic stimulation.

If exercise tachycardia is taken as the endogenous stimulus then a dose ratio of about 30 is equivalent to 100% blockade and a ratio of about 10 equivalent to 20% blockade. Several workers have used a single dose of isoprenaline (usually enough to increase the rate by 30-40 beats/minute) to test β-adrenergic blockade. Complete abolition of such a response in

our studies would imply a dose ratio of about 10, which corresponds to only 20% blockade of exercise tachycardia, and therefore at greater degrees of blockade the test is only qualitative.

The measurement of circulating levels of a drug is the only rational criterion for estimating dose, because both blood and plasma levels (and therefore the amount of drug reaching the target organ) vary widely after a fixed oral dose (Grant *et al.*, 1966; Shanks, 1966; Shand *et al.*, 1970). The present study has demonstrated a difference between the oral and intravenous routes of administration, showing that about three times the plasma levels are required to produce a given effect after intravenous than after oral administration (Fig. 1). An explanation of this finding is apparent from the work of Paterson *et al.* (1970), who found that a metabolite of propranolol, 4-hydroxypropranolol, could be detected after oral administration but not after intravenous administration, and, furthermore, 4-hydroxypropranolol has β-adrenergic blocking properties (Barrett, personal communication). Clearly, the contribution made by this active metabolite to the effects of orally administered propranolol will depend on the amounts present and its potency relative to the parent drug. In the two subjects studied Paterson *et al.* (1970) found 4-hydroxypropranolol to be present in about equal amounts to propranolol, and in animals it is about equipotent with propranolol. If these estimates were confirmed in man they would suggest that the plasma propranolol levels after intravenous administration should be about twice those giving the same effect after oral administration. This is in reasonable agreement with the ratio of 3 found in the present study.

Maximal reduction of exercise-induced tachycardia was found with plasma propranolol levels above 40-50 ng./ml. after oral administration and 100-150 ng./ml. when given intravenously, and it is tempting to speculate that if a given therapeutic effect is produced only at levels in excess of these, then it is not solely due to β-adrenergic blockade. It has been suggested that some effects of propranolol, such as heart failure, result from direct myocardial depression (or a quinidine-like) effect, though some doubt has been cast on this recently. In animal and human heart muscle at least 3-10 μg./ml. is required to show any change in the action potential in vitro (Davis and Temte, 1968; Coltart and Meldrum, 1970). This concentration is about 100-fold greater than those found after about 20 mg. intravenously which was required for complete β-blockade, a difference that would be magnified by any binding of propranolol to plasma proteins. It seems highly unlikely, therefore, that direct myocardial depression has been seen clinically after intravenous therapy, and supports the suggestion that heart failure results from the removal of the increased sympathetic drive that is required to maintain the cardiac output in certain patients (Chidsey *et al.*, 1965). This property would still be present with β-blocking drugs which lack quinidine-like effects, and the precipitation of heart failure may still be a potential hazard.

The question of whether an action other than β-blockade is present during continuous oral therapy is more difficult to answer. Certainly, the doses of propranolol required for adequate treatment of angina and hypertension can vary widely, and high doses of the order of 1-2 g./day have been used. Some variability in oral dose can be accounted for by the variation in the absorption of propranolol as parent drug, but from the data in the literature and from some unpublished observations plasma levels of greater than 40 ng./ml. should be attained by at most 160 mg./day and a significant degree of blockade should be present at lesser doses. It is possible that this might represent an underestimation in the outpatient setting. For example, it has recently been shown that, for phenytoin, outpatients appeared to be taking half the prescribed dose compared to when they were inpatients (Gibberd *et al.*, 1970).

The possibility that a physiological adaptation might occur during chronic therapy should be considered (Pritchard and Gillam, 1969). Also, the role of 4-hydroxypropranolol in the treatment of angina and hypertension is unknown. Clearly, further work on the plasma levels of propranolol associated with successful therapy of these conditions is required before the role of β-adrenergic blockade in such treatments can be fully assessed. There is a tendency to consider patients with angina or essential hypertension as single populations, but there is every possibility that only certain patients will respond to β-adrenergic blockade and that they represent a separate subgroup. Nevertheless, it remains a distinct possibility that a non-specific effect of propranolol could contribute to its efficacy, though it is unlikely that this would be a "quinidine-like" action, and other effects, including those on the central nervous system, merit further investigation.

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