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The effects of lower than conventional doses of oral nadolol on relative β_1/β_2-adrenoceptor blockade

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1 The aim of the present study was to evaluate the relative β_1/β_2 antagonist selectivity of the β-adrenoceptor blocker nadolol, in lower than conventional clinical doses.

2 Eight normal volunteers received single oral doses of either placebo (PL), nadolol 5 mg (N5), 20 mg (N20) or 80 mg (N80) in a single-blind, randomised crossover design. β_1-adrenoceptor antagonism was assessed by attenuation of exercise tachycardia, and β_2-adrenoceptor blockade by effects on salbutamol-induced chronotropic, hypokalaemic and finger tremor responses. The relative percentage attenuation of β_2 and β_1-mediated responses was calculated and expressed as β_2:β_1 selectivity ratios.

3 Nadolol produced dose-related reductions in exercise tachycardia in keeping with increasing β_1-adrenoceptor blockade; mean % reduction (95% CI) compared with placebo: N5 10.7 (6.6 to 14.8), N20 21.4 (17.3 to 25.4), N80 38.9 (34.8 to 42.9). However, even the lowest dose of nadolol (5 mg) produced almost complete blunting of β_2-mediated effects and significantly increased exercise hyperkalaemia; peak exercise hyperkalaemia (mmol l^{-1}) (means and 95% CI): PL 4.88 (4.68 to 5.07), N5 5.36 (5.17 to 5.55), N20 5.48 (5.28 to 5.67), N80 5.42 (5.22 to 5.61). β_2:β_1 selectivity ratios significantly increased as the dose of nadolol was reduced.

4 These data suggest that whereas in the clinical dose range nadolol behaves as a non-selective β-adrenoceptor antagonist, as the dose is reduced this drug demonstrates an increasing degree of selectivity for the β_2-adrenoceptor.

5 Low-dose oral nadolol may therefore be a useful selective β_2-adrenoceptor antagonist for research studies in man.

Keywords nadolol salbutamol β-adrenoceptor selectivity

Introduction

β-adrenoceptor antagonists have conventionally been classified into two main groups: 'non-selective' agents which block β_1 and β_2-adrenoceptors, and drugs which exhibit greater antagonism at one or other β-adrenoceptor subtype, being termed 'selective' for that receptor. It has however become evident that β-adrenoceptor subtype selectivity is a relative rather than an absolute property, in that drugs which are intrinsically selective for either the β_1 or β_2-adrenoceptor have been found to antagonise the other receptor subtype in a dose-dependent manner [1–3].

Whether the reverse occurs is not entirely clear; i.e. will a β-adrenoceptor blocking drug which is 'non-selective' polarise its effects in favour of one or other β-adrenoceptor subtype when used in a lower than conventional dose. There is some evidence to suggest that this may be the case with propranolol. Firstly, *in vitro* studies have demonstrated that propranolol has a 2–3 fold greater affinity for β_2 than β_1-adrenoceptors [4, 5]. Secondly, a low dose of propranolol (10 mg) produces a proportionately greater degree of antagonism of β_2 compared with β_1-adrenoceptor mediated responses *in vivo* [6].

If low dose oral propranolol does indeed behave as a

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relatively β_2-selective adrenoceptor antagonist, this might prove to be a useful pharmacological probe for dissecting out β_1 and β_2-mediated responses *in vivo*. However, since oral propranolol undergoes significant first-pass hepatic metabolism, we decided to investigate the properties of low dose oral nadolol which is hydrophilic and therefore exhibits more predictable pharmacokinetics.

Thus, the purpose of the present study was to evaluate the β_2:β_1-adrenoceptor selectivity of lower than conventional doses of oral nadolol. β_1-adrenoceptor blockade was assessed by reduction of maximal exercise tachycardia [6, 7], and β_2-adrenoceptor blockade by attenuation of chronotropic, finger tremor and hypokalaemic responses to the selective β_2-adrenoceptor agonist salbutamol [8, 9].

Methods

Subjects

Eight normal volunteers were studied with a mean age (s.e. mean) of 23 ± 3 years. Prior to entry into the study all subjects provided written informed consent and were required to have had a normal physical examination, full blood count, biochemical profile, urinalysis and 12-lead electrocardiogram. Exclusion criteria were a history of asthma, hypertension, cardiac arrhythmias, diabetes and thyroid disease, and no subjects were taking concurrent medication. Approval for the study was granted by the Tayside Medical Ethics Committee.

Protocol

Subjects attended the laboratory at the same time of day on each of the 4 study days, which were separated by weekly intervals. All had fasted for 4 h prior to attendance and physical exercise, nicotine and caffeine consumption were prohibited on study days. Treatments were given in a single-blind, randomised (Latin square), crossover design and 2.5 h prior to attendance subjects ingested single oral doses of either placebo (PL), nadolol 5 mg (N5), nadolol 20 mg (N20) or nadolol 80 mg (N80). On arrival subjects were weighed and an intravenous cannula was inserted into each antecubital fossa for the purposes of blood sampling and drug infusion. Blood was withdrawn for measurement of plasma nadolol concentrations at the time point 2.5 h after ingestion. Subjects then performed a standardised 3 min exercise step test [7] in order to achieve maximum exercise heart rate. Peak exercise heart rate (EHR) was recorded and immediately on completion of exercise venous blood was taken for the measurement of serum potassium (K). Subjects then rested supine for a 45 min recovery period. Baseline measurements of heart rate (HR) and postural finger tremor (Tr) were made and blood withdrawn for measurement of serum K and nadolol concentration. Subsequently an intravenous infusion of salbutamol sulphate ('Ventolin', Allen and Hanburys Ltd, Middlesex, UK) in 0.9% sodium chloride was given at a rate of 0.2 μg kg^{-1} min^{-1} for 30 min, and measurements of HR, Tr, K and plasma salbutamol concentrations were made at 10 min intervals throughout.

Measurements

For safety purposes, heart rate was continuously recorded throughout the study period and the mean heart rate at each time point calculated from the mean of five consistent R–R intervals. Postural finger tremor was measured by a previously validated method [10] with an accelerometer transducer (Entran Ltd, Ealing, UK) and computer-assisted spectral analysis using autocovariance. The total tremor power >20 Hz was calculated from the mean of five consistent recordings and used for analysis. All blood samples were analysed in batches at the end of the study and assayed in duplicate. Serum potassium was measured by flame photometry (IL 943 analyser; Instrumentation Laboratory Ltd, Warrington, UK). The normal reference range for serum potassium in our laboratory is 3.5–5.0 mmol l^{-1}. Plasma drug concentrations were analysed by high performance liquid chromatography using pindolol as an internal standard for the nadolol assay. The respective coefficients of variability for analytical imprecision within and between assays were as follows: nadolol (2 ng ml^{-1}) 12.4% and 15.9%; nadolol (50 ng ml^{-1}) 4.4% and 6.8%; salbutamol (7.5 ng ml^{-1}) 4.0% and 6.7%.

Statistical analysis

All data were analysed using a 'Statgraphics' software package (STSC Software Publishing Group, Bethesda, USA). Repeated measures multifactorial analysis of variance was used to compare the effects of treatments on the time profiles of each variable. Bonferroni multiple range testing with confidence limits set at 95% was used to identify time points where differences between treatments were significant. All results are calculated as changes from baseline and are expressed in the text as means and 95% confidence intervals. Plasma drug concentrations are given as means ± s.e. mean. Effects of treatments on exercise tachycardia are expressed as a percentage reduction compared with the maximum heart rate obtained with placebo. Similarly, treatment effects on salbutamol-induced chronotropic, hypokalaemic and finger tremor responses are expressed as a percentage reduction compared with the maximal effect obtained with placebo. Salbutamol effects are given at the time point of peak response, 30 min after the start of the infusion. β_2:β_1 selectivity ratios for each treatment were calculated by comparing the percentage reduction of each salbutamol-induced response (β_2) with the percentage reduction of exercise tachycardia (β_1). A probability value of $P < 0.05$ was considered as being of significance for all statistical tests, and hence for clarity individual P values are not given where a result is stated in the text to be significant. The use of eight subjects allowed a 0.3 mmol l^{-1} change in the end-point of serum potassium to be detected with 80% power.

Results

Plasma nadolol concentrations

Dose-related increases in plasma nadolol concentrations (ng ml^{-1}) occurred and were as follows (mean ± s.e. mean): N5 (4.4 ± 0.6); N20 (14.8 ± 1.6); N80 (81.8 ± 21.8). No significant changes occurred in plasma nadolol concentration between the beginning and end of the study on each day.

Plasma salbutamol concentrations

No significant differences occurred in plasma salbutamol concentrations between each of the study days. Plasma salbutamol concentrations (ng ml^{-1}) 30 min after the start of the infusion are given for each treatment day and were as follows (means ± s.e. mean): PL (6.46 ± 0.28); N5 (6.91 ± 0.42); N20 (6.80 ± 0.38); N80 (7.00 ± 0.26).

Exercise tachycardia (Figure 1)

Nadolol produced dose-related reductions in exercise-tachycardia compared with PL, with significant differences occurring between each of the doses used. % reduction of EHR cf PL (means and 95% CI): N5 10.7 (6.6 to 14.8), N20 21.4 (17.3 to 25.4), N80 38.9 (34.8 to 42.9).

Peak exercise hyperkalaemia (Figure 2)

All doses of nadolol produced a significant increase in peak exercise hyperkalaemia compared with PL. Peak exercise K (mmol l^{-1}) (means and 95% CI): PL 4.88 (4.68 to 5.07), N5 5.36 (5.17 to 5.55), N20 5.48 (5.28 to 5.67), N80 5.42 (5.22 to 5.61). No significant differences were seen between each dose of nadolol used. Mean differences (95% CI) compared with N5 were: N20 −0.12 (−0.64 to 0.40), N80 −0.06 (−0.58 to 0.46).

Salbutamol-induced responses (Figure 3)

The effects of treatments on salbutamol-induced chronotropic, hypokalaemic and finger tremor responses showed a similar pattern. A significant increase in each response occurred after PL and this effect was almost completely blocked in each case by the 5 mg dose of nadolol (see Table 1). No significant differences were found between each dose of nadolol. Mean differences and the associated 95% CI, compared with N5, were as follows: HR (beats min^{-1}): N20 0.1 (−7.8 to 8.0), N80 5.0 (−2.9 to 12.9). K (mmol l^{-1}): N20 −0.14 (−0.6 to 0.32), N80 0.08 (−0.38 to 0.54), Tr (mg^2 s^{-1}): N20 91 (−814 to 995), N80 99 (−806 to 1003).

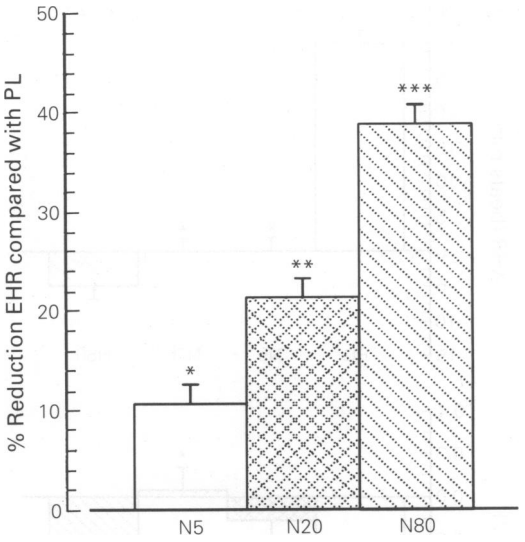

Figure 1 Effects of single oral doses of nadolol 5 mg (N5), 20 mg (N20) and 80 mg (N80) on reduction of exercise tachycardia compared with placebo (PL). *$P < 0.05$ compared with PL, **$P < 0.05$ compared with N5, ***$P < 0.05$ compared with N20.

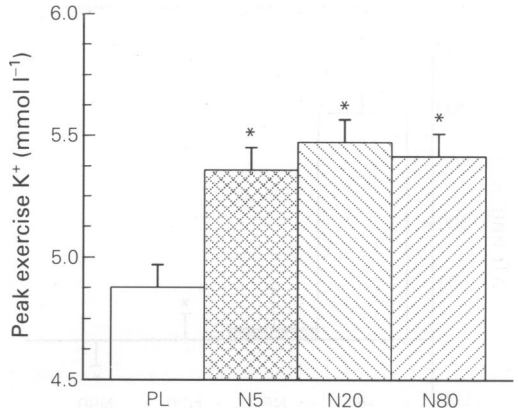

Figure 2 Effects of single doses of nadolol on peak exercise hyperkalaemia (abbreviations as in Figure 1).

β_2:β_1 selectivity ratios (Figure 4)

The effects of nadolol on β_2:β_1 selectivity ratios were similar whether salbutamol-induced chronotropic,

Table 1 Salbutamol-induced chronotropic (HR), hypokalaemic (K) and finger tremor (Tr) responses after pretreatment with placebo (PL), nadolol 5 mg (N5), 20 mg (N20) and 80 mg (N80). Data expressed as mean changes and 95% CI

	PL	*N5*	*N20*	*N80*
Δ HR (beats min^{-1})	30.0 (26.0 to 33.9)*	0.1 (−3.8 to 4.1)	0 (−4.0 to 4.0)	−4.9 (−8.8 to −0.9)
Δ K (mmol l^{-1})	−0.98 (−1.19 to −0.76)*	−0.11 (−0.33 to 0.11)	0.03 (−0.19 to 0.25)	−0.19 (−0.41 to 0.03)
Δ Tr (mg^2 s^{-1})	1952 (1498 to 2406)*	93 (−361 to 548)	3 (−451 to 457)	−5 (460 to 449)

*$P < 0.05$ compared with baseline.

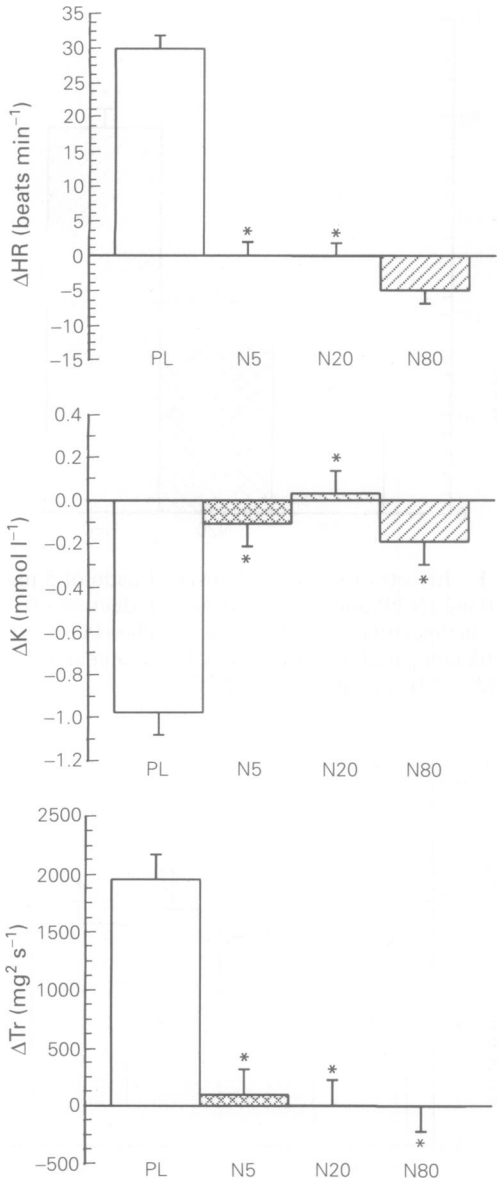

Figure 3 Effects of nadolol on salbutamol-induced a) chronotropic (HR), b) hypokalaemic (K) and c) finger tremor (Tr) responses (abbreviations as in Figure 1).

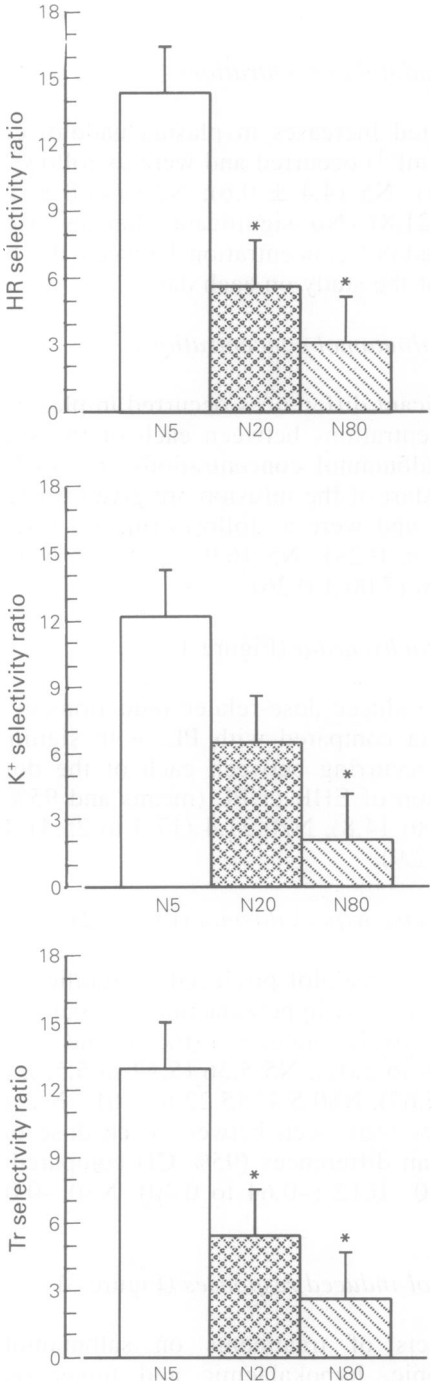

Figure 4 β_2:β_1 selectivity ratios for the effects of nadolol 5 mg (N5), 20 mg (N20) and 80 mg (N80) for: a) chronotropic, b) hypokalaemic (K) and c) finger tremor (Tr) responses. *$P < 0.05$ compared with N5.

hypokaalemic or finger tremor responses were considered. As the dose of nadolol was increased, in each case a significant dose-related reduction in the β_2:β_1 selectivity ratio occurred. Means and 95% CI are given.

HR selectivity ratio: N5 14.36 (8.6 to 19.86), N20 5.66 (0.16 to 11.16), N80 3.11 (−2.39 to 8.61).

K selectivity ratio: N5 12.18 (6.92 to 17.43), N20 6.51 (1.26 to 11.76), N80 2.10 (−3.15 to 7.35).

Tr selectivity ratio: N5 12.96 (8.73 to 17.20), N20 5.48 (1.24 to 9.71), N80 2.65 (−1.58 to 6.88).

Discussion

The results of the present study show that whereas a reduction in the dose of nadolol to lower than conventional levels is associated with a progressive decline in β_1-adrenoceptor blockade, β_2-antagonism is maintained and is almost maximal even at the lowest dose of 5 mg; i.e. a gradual shift towards selective β_2-adrenoceptor blockade occurs as the dose is reduced. This suggests that as the dose of nadolol is progressively reduced, there is a relatively flat dose-response curve for β_2-adrenoceptor blockade, but a steep dose-response for β_1-adrenoceptor blockade *in vivo*. Nadolol therefore appears to exhibit a greater affinity for β_2 than β_1-receptors. This phenomenon has to a lesser extent been demonstrated with propranolol, both *in vitro* and *in vivo* [4–6], although to our knowledge no previous studies have

been performed using nadolol. The findings of the current study with nadolol are substantiated by previous data using isoprenaline, a non-selective β_1/β_2-adrenoceptor agonist [11]. It was shown that a 5 mg dose of nadolol produced almost complete β_2-adrenoceptor blockade in terms of the finger tremor response to isoprenaline, but had no effect on the systolic blood pressure response which is predominantly β_1-mediated [12]. Higher doses of nadolol (20 mg and 80 mg) produced complete blunting of both the β_1 and β_2-mediated effects of isoprenaline. Taken together, these studies therefore imply that nadolol is only non-selective at doses used in clinical practice (40–160 mg), but at lower doses (5 mg) it produces preferential β_2-adrenoceptor blockade.

In order to understand more clearly the results of this study, it is important to consider the methodology we have used to evaluate β_1- and β_2-adrenoceptor blockade *in vivo*. β_1-adrenoceptor antagonism was assessed by attenuation of exercise tachycardia [6, 7], since it has been shown that β_1-adrenoceptor antagonists produce dose-related reductions in this response [1, 2, 13], whereas selective β_2-adrenoceptor blockade has no effect [6, 12]. In the present study, increasing doses of nadolol produced a progressive reduction in exercise heart rate in keeping with β_1-adrenoceptor antagonism, and significant differences in response were apparent at each dose increment.

β_2-adrenoceptor antagonism was assessed by studying the effects of treatments on the chronotropic, hypokalaemic and finger tremor responses to the selective β_2-adrenoceptor agonist salbutamol [8, 9]. In each case the pattern of the response was similar. Salbutamol produced significant increases in heart rate and finger tremor, and a significant fall in serum potassium in keeping with β_2-receptor stimulation. Chronotropic responses were considered to be β_2-mediated, since there is no good evidence to suggest that salbutamol loses β_2-adrenoceptor selectivity at this dose. All doses of nadolol completely blocked these responses, demonstrating that near-maximal β_2-adrenoceptor blockade occurred even using the lowest dose of nadolol (5 mg). In addition, it has previously been shown that drugs which block β_2-adrenoceptors potentiate the hyperkalaemic response to exercise [2, 14, 15], and this was also used as an additional index of β_2-adrenoceptor antagonism. In this respect, all doses of nadolol used in the present study produced a significant increase in peak exercise hyperkalaemia compared with placebo. A dose-response effect was not seen, implying that even the lowest dose of nadolol (5 mg) produced near-maximal β_2-adrenoceptor blockade, and this is in keeping with its effects on the other β_2-mediated responses previously discussed.

Having demonstrated individually the β_1 and β_2-blocking effects of each dose of nadolol, it is now important to consider the concept of β-adrenoceptor subtype selectivity and how this should be expressed. Several β-adrenoceptor antagonists such as atenolol, bisoprolol and betaxolol are known to block β_1 to a much greater degree than β_2-receptors and are termed β_1-selective. However, selectivity is a relative rather than an absolute property, since it has been shown that increasing doses of a β_1-selective antagonist produces dose-related β_2-adrenoceptor blockade [1, 2, 8, 9] and that higher doses of the β_2-adrenoceptor selective antagonist ICI 118,551 inhibit exercise tachycardia [3]. Although dose-related β_2-adrenoceptor blockade becomes evident, drugs such as atenolol, even in large doses do not antagonise β_2-receptors to the same degree as for example, do propranolol or nadolol, and in this respect they do not become truly 'non-selective' [8].

In pharmacological terms, β-adrenoceptor subtype selectivity refers to the ratio of β_2:β_1 antagonism. In the present study we therefore compared the effects of nadolol on the percentage reduction of salbutamol-induced responses (β_2-adrenoceptor blockade), with its effects on reduction of exercise tachycardia (β_1-adrenoceptor blockade), and the results were expressed as a β_2/β_1 selectivity ratio for each variable. A similar pattern of response occurred for chronotropic, hypokalaemic and finger tremor effects, in that increasing doses of nadolol produced a significant and progressive fall in the β_2/β_1 selectivity ratio.

In summary, we have evaluated the β_1 and β_2-adrenoceptor antagonism produced by nadolol across a 16-fold dose range (5–80 mg), and have shown that this drug displays selective β_2-adrenoceptor blockade at doses lower than those used in clinical practice. Low-dose nadolol (5 mg) may therefore be a useful β_2-selective antagonist for use as a research tool.

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