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An evaluation of the α-adrenoceptor antagonism produced by SK&F 86466 in healthy normotensive males

R. F. SCHAFERS, H. L. ELLIOTT, C. A. HOWIE & J. L. REID
University Department of Medicine and Therapeutics, Stobhill General Hospital, Glasgow, G21 3UW

SK&F 86466 is a novel, potent α-adrenoceptor antagonist which, in animal experiments, is reported to show a high selectivity for α_2-adrenoceptors at both pre- and post-junctional sites. The effects of two intravenous doses of 80 and 200 μg kg^{-1} of SK&F 86466 were assessed in a placebo-controlled, double-blind, randomised study in eight young, healthy, normotensive males. Two indices of α-adrenoceptor activity were investigated: i) Pressor responsiveness to the relatively selective α_1-adrenoceptor agonist phenylephrine and to the preferential α_2-adrenoceptor agonist α-methylnoradrenaline. ii) Circulating levels of noradrenaline. SK&F 86466 at a dose of 200 μg kg^{-1} produced rightward shifts of the pressor dose-response curves to both agonists: a 1.4 fold shift for phenylephrine (P = 0.023) and a 1.6 fold shift for α-methylnoradrenaline (P = 0.051). Erect plasma noradrenaline sampled at 105 min into the infusion was significantly increased from 2.9 to 5.0 nmol l^{-1} by SK&F 86466 200 μg kg^{-1} (P = 0.002). The change in the phenylephrine responses indicates post-junctional α_1-adrenoceptor blockade and the rise in noradrenaline is consistent with pre-junctional α_2-adrenoceptor antagonist activity. Overall the results of this study suggest that SK&F 86466, at a dose of 200 μg kg^{-1}, causes both α_1- and α_2-adrenoceptor antagonism in human subjects.

Keywords SK&F 86466 α_2-adrenoceptor antagonist pressor responses

Introduction

There is some evidence that pre-junctional α_2-adrenoceptors are involved in the regulation of noradrenaline release in man (Brown *et al.*, 1985; Jie *et al.*, 1987) and that post-junctional α_2-adrenoceptors may contribute to the regulation of human peripheral vascular tone (van Brummelen *et al.*, 1986). In addition it has been reported that post-junctional α_2-adrenoceptor mediated vasoconstriction is enhanced in essential hypertension (Bolli *et al.*, 1984).

SK&F 86466 (6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine hydrochloride) is a recently developed drug which has been characterised by *in vitro* studies in various animal tissues as a potent and selective antagonist at both pre- and post-junctional α_2-adrenoceptors (Hieble *et al.*, 1986).

This study was designed to evaluate the nature and extent of the α-adrenoceptor antagonist activity of SK&F 86466 in man.

Methods

Subjects

Eight healthy, normotensive males, aged 22 to 30 years, gave written informed consent for participation in the study which had received prior approval by the local Research and Ethics Committee.

Correspondence: Dr H. L. Elliott, University Department of Medicine and Therapeutics, Stobhill General Hospital, Glasgow, G21 3UW

Study design

In a randomised, double-blind, crossover study, with study days at weekly intervals, the effects of three treatments were evaluated: placebo (vehicle) and two intravenous (i.v.) doses of 80 and 200 $\mu g\ kg^{-1}$ of SK&F 86466. Each treatment was administered as a constant rate infusion (835 $\mu l\ min^{-1}$) over 120 min after at least 30 min rest supine.

Blood pressure and heart rate Blood pressure (BP) and heart rate (HR), supine and erect, were measured at intervals throughout each study day with a semiautomatic sphygmomanometer (Datascope Accutorr 1 and 1A).

Pressor responsiveness Pressor responses to incremental, intravenous infusions of the relatively selective α_1-adrenoceptor agonist phenylephrine and of the preferential α_2-adrenoceptor agonist α-methylnoradrenaline were performed on each study day at 30 and 105 min after the end of the drug infusion. Pressor responsiveness was expressed as the PD_{15} ($\mu g\ kg^{-1}\ min^{-1}$)—the pressor dose of agonist required to raise systolic blood pressure by 15 mm Hg (Sumner & Elliott, 1987) (Figure 1). The sequence of agonist administration was randomly allocated but kept constant for each individual on all study days.

Plasma noradrenaline Blood was sampled from a forearm vein for plasma noradrenaline on four occasions during each study day: at 30 min (supine) and at 105 min (erect) after starting the treatment infusion and then immediately before the two pressor response studies (supine). At 105 min, erect posture was chosen as a physiological stimulus in order to investigate the effect of SK&F 86466 on endogenous noradrenaline release in a setting of increased sympathetic nervous activity. Noradrenaline was assayed by high performance liquid chromatography with electrochemical detection (h.p.l.c.-ED) (Howes *et al.*, 1985).

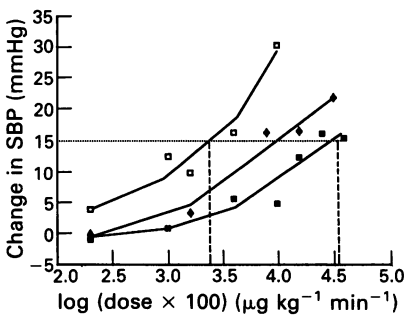

Figure 1 Individual (subject 7) pressor dose-response to α-methylnoradrenaline fitting a quadratic model following placebo (□), SK&F 86466 80 $\mu g\ kg^{-1}$ (◆) and 200 $\mu g\ kg^{-1}$ (■).

Statistics

Statistical evaluation was by repeated measures analysis of variance (ANOVA). Bonferroni confidence intervals (CI) were constructed as appropriate.

Results

Blood pressure and heart rate

SK&F 86466 had no significant effects on blood pressure and heart rate, supine or erect, during the infusion of drug or placebo.

Pressor responsiveness

Following placebo the mean PD_{15} values were 1.65 + 0.34 for phenylephrine and 0.31 + 0.10 $\mu g\ kg^{-1}\ min^{-1}$ for α-methylnoradrenaline and there were no significant changes in the pressor responses following 80 $\mu g\ kg^{-1}$ SK&F 86466 for either phenylephrine or α-methylnoradrenaline, although PD_{15} values were slightly increased to 2.15 ± 0.86 and 0.44 ± 0.18 $\mu g\ kg^{-1}\ min^{-1}$ respectively.

After 200 $\mu g\ kg^{-1}$ SK&F 86466, the PD_{15} for phenylephrine increased significantly from 1.65 ± 0.34 to 2.24 ± 0.66 $\mu g\ kg^{-1}\ min^{-1}$ ($P = 0.023$) and correspondingly, the PD_{15} for α-methylnoradrenaline increased from 0.31 ± 0.10 to 0.49 ± 0.23 $\mu g\ kg^{-1}\ min^{-1}$ ($P = 0.051$) (Figure 2). The mean differences ($\mu g\ kg^{-1}\ min^{-1}$) between placebo and SK&F 86466 were −0.59 for phenylephrine (95% CI: −1.13 to −0.04) and −0.18 for α-methylnoradrenaline (95% CI: −0.36 to 0.004).

Plasma noradrenaline

Following placebo, plasma noradrenaline was 2.23 ± 0.77 $nmol\ l^{-1}$ at 30 min (supine) and 2.93 ± 1.33 $nmol\ l^{-1}$ at 105 min (erect) into the infusion. For the 30 min measurement there were no changes attributable to SK&F 86466 with values of 1.90 ± 0.38 (80 $\mu g\ kg^{-1}$) and 2.15 ± 0.58 (200 $\mu g\ kg^{-1}$). For the 105 min (erect) plasma noradrenaline there was no significant change with the 80 $\mu g\ kg^{-1}$ dose but with 200 $\mu g\ kg^{-1}$ SK&F 86466 there was a significant increase ($P = 0.002$) to 5.00 ± 0.72 $nmol\ l^{-1}$. The 95% confidence interval for this difference was −3.24

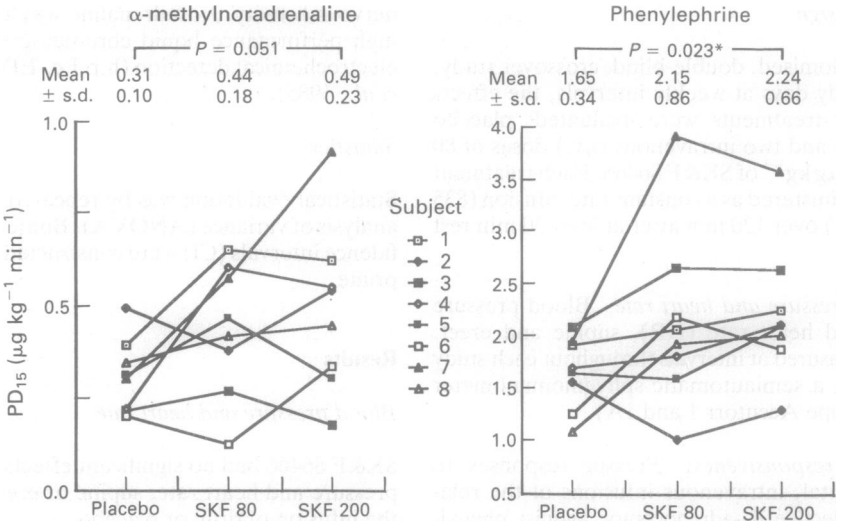

Figure 2 The influence of SK&F 86466 at a dose of 80 and 200 μg kg^{-1} on pressor responsiveness to α-methylnoradrenaline and phenylephrine. Dot plots of PD_{15} values (systolic blood pressure).

to −0.91 nmol l^{-1}, with a mean difference of −2.08 nmol l^{-1}.

As for the 30 min measurement no effect of SK&F 86466 was observed on supine noradrenaline levels prior to the two pressor infusions.

Discussion

Pressor responsiveness (i.e. the increase in blood pressure in response to systemic intravenous administration of an agonist drug) using the relatively selective α_1-adrenoceptor agonist, phenylephrine, has proved a useful and valuable tool for the investigation of α_1-adrenoceptor function in humans (Sumner & Elliott, 1987; Sumner *et al.*, 1987).

Interest in the clinical pharmacology of α_2-adrenoceptor blocking drugs has been stimulated recently by the development of a growing number of compounds which demonstrate a highly selective α_2-adrenoceptor blockade in animal tissues.

α-methylnoradrenaline is a relatively selective α_2-adrenoceptor agonist (Ruffolo, 1984) which does not cross the blood brain barrier. It does therefore appear to be a suitable choice for the study of peripheral vascular α_2-adrenoceptor function if administered as a systemic pressor infusion. There are, however, a number of potential compounding factors which require consideration for an unbiased interpretation of its systemic pressor effects.

Firstly, α-methylnoradrenaline also shows a weak β-adrenoceptor stimulating i.e. vasodilating effect (Ahlquist, 1948) which may therefore partially counteract an α_2-adrenoceptor mediated vasoconstriction. However, a weak β-adrenoceptor agonist action has similarly been described for phenylephrine (Flavahan & McGrath, 1981). Consequently, an evaluation of the relative α_2- *vs* α_1-selectivity of an α-adrenoceptor blocking compound like SK&F 86466 by comparing the response to pressor infusions of both phenylephrine and α-methylnoradrenaline should not be unduly affected by a weak β-adrenoceptor agonist property which is owned by both pressor agonists.

Secondly, although there is now convincing evidence that post-junctional vascular α_2-adrenoceptors are involved in the regulation of vascular tone in humans (Van Brummelen *et al.*, 1986) stimulation of the 'classical' vascular α_1-adrenoceptors appears to remain the major determinant of systemic vascular resistance. It is therefore conceivable that, at least at high infusion rates, α-methylnoradrenaline loses its relative α_2- *vs* α_1-selectivity and may also stimulate vascular α_1-adrenoceptors.

Realising these potential limitations of systemic pressor infusions of α-methylnoradrenaline the 1.6 fold shift in the pressor response curve for α-methylnoradrenaline following the 200 μg kg^{-1} dose of SK&F 86466, which narrowly fails to reach conventional levels of statistical significance, is suggestive of only a very modest degree of post-junctional α_2-adrenoceptor-blockade. Similar evidence of modest post-junctional α_2-adrenoceptor antagonist activity was obtained

by Brown *et al.* (1988), who investigated the effects of orally administered SK&F 86466 on clonidine-induced reduction of skin blood flow.

More unequivocally, the highly significant elevation in erect plasma noradrenaline at the end of the 200 $\mu g\ kg^{-1}$ infusion, in the absence of a concomitant fall in blood pressure, is consistent with an antagonist action at pre-junctional α_2-adrenoceptors and again is consistent with the findings of Brown *et al.* (1988) and of de Mey *et al.* (1989). A similar increase in circulating noradrenaline was reported for the α_2-adrenoceptor antagonist, idazoxan (Elliott *et al.*, 1983) and a recent investigation by Jie *et al.* (1987) has lent further support for the concept that pre-junctional α_2-adrenoceptors are involved in the regulation of noradrenaline release *in man*.

The modest, but significant, reduction in phenylephrine pressor responsiveness after 200 $\mu g\ kg^{-1}$ SK&F 86466, which was present in seven out of eight subjects, additionally suggests that the drug also has α_1-adrenoceptor activity. This is at variance with the reported α_2-selectivity in animal experiments (Hieble *et al.*, 1986). There are several possible explanations for this apparent discrepancy in the relative extent of the α_1- and α_2-selectivities in man compared with those of the animal investigations. Firstly there is evidence that α_2-adrenoceptors display significant species differences (Bylund, 1985) and this study may simply illustrate the problem of extrapolating from *in vitro* effects in animals to *in vivo* effects in man. Secondly, and irrespective of sub-type selectivity, there is evidence that SK&F 86466 is less potent than other known α_2-adrenoceptor antagonists at human α_2-adrenoceptors. For example, it displays a lower potency and affinity than either yohimbine or idazoxan for α_2-adrenoceptors in isolated human fat cells (Galitzky *et al.*, 1988). Furthermore, using the same experimental approach as in this study, Elliott & Reid (1983) were able to demonstrate that idazoxan produced a marked and highly significant decrease in the pressor responsiveness to α-methylnoradrenaline. Finally, it is possible that the doses chosen for this study may not have been ideal for revealing selective post-junctional α_2-adrenoceptor blockade such that the apparent discrepancy may be dose-related.

In conclusion, these results suggest that SK&F 86466 at an intravenous dose of 200 $\mu g\ kg^{-1}$ causes both α_1- and α_2-adrenoceptor antagonism in human subjects.

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