INVESTIGATION OF A COMBINED ARTERIOLAR DILATOR AND β -ADRENOCEPTOR ANTAGONIST (SK & F 92657) IN THE PERIPHERAL VESSELS OF MAN

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- 1 SK & F 92657 is a new compound which in animals has both precapillary vasodilator and β -adrenoceptor blocking activity. The effects of the drug on forearm blood flow and dorsal hand vein distensibility have been studied in healthy volunteers.
- 2 Forearm blood flow was measured by venous occlusion plethysmography. The drug was infused directly into a brachial artery. The effects of SK & F 92657 on resting flow and on the dose-response relationship for intra-arterial isoprenaline were studied.
- 3 Venous distensibility was measured in the large veins on the back of the hand. The drug was infused directly into a selected vein and its effects on resting tone and on the responses to noradrenaline and to isoprenaline were investigated.
- 4 Intra-arterial infusion of SK & F 92657 over 2 min caused a dose-dependent increase in forearm blood flow which developed gradually to reach a maximum after approximately 40 min. Infusion into dorsal hand veins at 100 ng/min and 500 ng/min had no dilator effect in veins precontracted with noradrenaline. At higher doses SK & F 92657 potentiated the constrictor effect of noradrenaline.
- 5 In both veins and arteries SK & F 92657 attenuated the dilator effects of isoprenaline. The drug had no effect on the venodilator effects of bradykinin, histamine or sodium nitroprusside.
- 6 The pattern of response of these vessels to locally infused SK & F 92657 is exactly that expected of a drug combining the properties of hydralazine-like vasodilatation and β -adrenoceptor blockade. Furthermore, in the arterioles, both properties were manifest at the same dose.

Introduction

Both β -adrenoceptor blocking agents and vasodilators are effective in the treatment of hypertension and advantages may be gained by using the two types of drug in combination (Zacest, Gilmore & Koch-Weser, 1972). However, complex combinations of tablets may reduce patient compliance (Taylor, Sackett, Haynes, Johnson, Gibson & Roberts 1978) and the development of a single compound possessing both pharmacological actions may permit useful simplification of therapeutic regimes in hypertension. Animal studies have shown that SK & F 92657 (dl-3-(2-(3-t-butylamino-2-hydroxypropoxy) phenyl-6-hydrazinopyridazine) (Figure 1) is such a compound in that it causes both precapillary vasodilatation and β -adrenoceptor blockade (Taylor, Roe & Slater,

1979). We have examined the effects of local infusions of SK & F 92657 in the forearm arterial bed and dorsal hand veins of healthy human volunteers.

Methods

Experiments were performed on healthy volunteers (17 male, 9 female, aged 20-38 years) each of whom

Figure 1 Structural formula of SK & F 92657; dl-3-(2-(3-t-butylamino-2-hydroxypropoxy) phenyl-6-hydrazinopyridazine).

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gave their informed consent to the study. Prior approval was obtained from the Ethical Committee of St George's Hospital. Subjects were studied at rest in the supine position. Room temperature varied between 22°C and 26°C on different occasions, but remained essentially constant during each experiment.

Drugs

Immediately prior to each experiment drugs were diluted to the required concentrations using 0.15M sodium chloride solution. Concentrations of drugs were chosen so as to be locally effective, without causing systemic effects. To prevent oxidation, ascorbic acid was added to noradrenaline and isoprenaline solutions to a final concentration approximately equivalent to that of the agonist drug.

Forearm arterial studies

Forearm blood flow was measured by venous occlusion plethysmography (Whitney, 1953) using temperature-compensated mercury-in-rubber strain gauges, applied to both forearms and connected to a Lectromed amplifier and strip-chart recorder. During measurement, wrist cuffs were inflated to a suprasystolic pressure (180–200 mm Hg); collecting cuffs around the upper arms were inflated to a pressure of 40 mm Hg for 10 s in every 15.

A 26 SWG needle was introduced under local anaesthesia (1% lignocaine) into a brachial artery in the antecubital fossa. Drugs were infused through this needle via a fine polythene cannula using a Harvard Apparatus constant rate infusion pump. The opposite arm served as a control.

In our initial series of experiments, the response to a 2 min infusion of SK & F 92657 (400 µg total dose) was followed for 30–90 min. In six subjects a second 2 min infusion of SK & F 92657 (1600 µg total dose) was given and the response was followed for a further 30–40 min.

The initial experiments showed that the dilator effect of SK & F 92657 was delayed. It was therefore possible, in a second series of experiments, to use the latent period after infusion of the first dose and before onset of its dilator action to examine possible β -adrenoceptor blockade by the drug. A dose-response relationship for the dilator effect of intra-arterial isoprenaline was firstly determined by infusing isoprenaline solution at different rates (25–100 ng/min) increased in logarithmic fashion, and measuring forearm blood flow during the third minute of infusion at each dose. Forearm flow was then allowed to return to initial levels. SK & F 92657, 400 µg was then infused over 2 min and the dose-response relationship for isoprenaline was deter-

mined again immediately afterwards. Saline was then infused and further measurements of forearm blood flow were made at intervals over 40 min.

Dorsal hand vein studies

Drugs were infused into a selected large dorsal hand vein via a 23 SWG butterfly needle, using a Harvard Apparatus constant rate infusion pump. Measurements of vein diameter were made at a point 5–10 mm downstream from the needle tip, using a modification of a previously described technique (Nachev, Collier & Robinson, 1971). Changes in vein size were detected by a lever resting on the summit of the vein and attached to a Harvard Apparatus displacement transducer, connected to a Lectromed amplifier and strip chart recorder. The hand veins were distended by maintaining a constant pressure of 25 mm Hg in a cuff around the upper arm. This congesting cuff was deflated at intervals, allowing the veins to empty. The displacement of the lever registered the change in vein size between the congested and the empty state; this change was taken as an index of vein distensibility at the congesting pressure applied.

All drugs were infused at a rate of 0.25 ml/min for 5 min at each concentration. The effect of SK & F 92657 (2 and 4 µg/min) on the relaxed vein was studied by measuring vein distensibility before infusion of the drug and at intervals over the following 40 min. In the same subjects the constrictor effect of noradrenaline (25 ng/min) was also examined before and after this dose of SK & F 92657.

A separate series of experiments was performed to assess the β -adrenoceptor blocking activity of SK & F 92657. A selected vein was constricted by a continuous infusion of noradrenaline (6.25–100 ng/min). A cumulative dose-response relationship for the dilatation caused by simultaneous infusion of isoprenaline (25–800 ng/min) into the precontracted vessel (Collier, Nachev & Robinson, 1972) was determined before and after the infusion of SK & F 92657 (500 ng/min).

The effect of SK & F 92657 (500 ng/min) on the dilator actions of histamine (10–80 ng/min), sodium nitroprusside (1–8 ng/min) and bradykinin (10–20 ng/min) was also studied.

Results

Forearm arterial studies

When infused into the brachial artery over 2 min SK & F 92657 (400 µg) caused a mean ($\pm$ s.e. mean) increase in forearm blood flow of 59% $\pm$ 14 (range 0–146%) at the 40th minute after infusion in 10 experiments. This effect did not begin for at least 10 min

after the infusion of SK & F 92657 had been completed and the increase in blood flow took 40–70 min to plateau, although the major increase took place by the 40th minute (Figure 2). In 1 experiment in which flows were followed until the response waned, the response had fallen to 50% of maximum by the 90th minute. In 6 experiments infusion of a second, larger dose of SK & F 92657 (1600 μg over 2 min) caused a further increase in forearm blood flow to $403\% \pm 81\%$ (111–630%), which began immediately and increased progressively over the 40 min following the

Figure 2 Effect of a 2 min intra-arterial infusion of SK & F 92657 (400 μg) on forearm blood flow in two subjects.

infusion. The response to increasing doses of SK & F 92657 in one subject is shown in Figure 3.

Figure 3 Effect on forearm blood flow of intra-arterial infusion of SK & F 92657 400 μg & 1600 μg (each dose given over 2 min) in one subject.

In the latent period after infusion of the lower dose of SK & F 92657 and before the onset of its own dilator effect, there was a 2.8 to 14-fold (mean 6.8-fold) attenuation of the dilator effect of isoprenaline (5 experiments) (Figure 4). When isoprenaline infusion ceased, forearm blood flow initially fell towards resting levels, and then increased again as the dilator effect of SK & F 92657 developed (Figure 5).

Figure 4 Individual log dose-response curves to isoprenaline before (●) and after (○) a 2 min intra-arterial infusion of 400 μg SK & F 92657 in five different subjects.

Figure 5 Schematic diagram showing the effect of SK & F 92657 (400 μ g i.a.) on the response of forearm blood flow to intra-arterial isoprenaline. When the second isoprenaline infusion ceased forearm flow fell towards resting levels and then increased again as the delayed dilator action of SK & F 92657 developed. The dotted lines indicate the predicted responses to SK & F 92657 and isoprenaline if each drug were given alone; the observed response, reflecting summation of the two effects, is shown by the full line.

Dorsal hand vein studies

No venodilator effect was seen when SK & F 92657 was infused into a dorsal hand vein. In a dose of 500 ng/min (15 experiments) it had no effect on the size of the noradrenaline-constricted vein. In 2 experiments, larger doses (2 and 4 μ g/min) potentiated the constrictor effect of noradrenaline; this potentiation persisted for at least 40 min after SK & F 92657 infusion.

Infusion of SK & F 92657 (500 ng/min) over 5 min caused 9 to 25-fold attenuation of the dilator action of isoprenaline-constricted veins in 9 experiments (Figure 6).

A lower dose of SK & F 92657 (100 ng/min) caused only a 2-fold attenuation of isoprenaline dilatation in the subject showing an 18-fold attenuation by 500 ng/min, infused into the same vein on a separate occasion.

Attenuation of the effect of isoprenaline persisted for at least 80 min after SK & F 92657 (1 experiment). Half the original potency of isoprenaline was regained after 70 min.

In contrast to this observed blockade of isoprenaline, SK & F 92657 (500 ng/min) had no effect on the dilator action of histamine (5 experiments), sodium nitroprusside (2 experiments) or bradykinin (1 experiment).

Figure 6 Log dose-response curves to isoprenaline in the veins before (●) and after (○) a 5 min i.v. infusion of SK & F 92657 at 500 ng/min in 9 experiments. (Each point shows the mean $\pm$ 1 s.e. mean).

Discussion

The actions of SK & F 92657 observed in human forearm arterioles and dorsal hand veins are those which would be expected of a combined vasodilator and β -adrenoceptor blocking agent. Vasodilatation in response to the drug was unusual, in being both arterioselective and of delayed onset. This pattern of activity has been seen previously only with hydralazine (Collier, Lorge & Robinson, 1978). Comparison of this effect of SK & F 92657 with that of hydralazine suggests that the potency of SK & F 92657 is approximately half that of hydralazine on a weight for weight basis. However, since the molecular weight of SK & F 92657 is approximately twice that of hydralazine, their potency on a molar basis is approximately equal.

β -adrenoceptor blockade by SK & F 92657 was confirmed by showing attenuation of the dilator action of isoprenaline in both veins and arterioles. Furthermore the parallel shift to the right of the dose response curve suggests that the blockade was competitive.

Specificity was shown in the veins since at the doses given there was no attenuation of the venodilator response to other substances, nor of the constrictor response to noradrenaline. In fact, SK & F 92657 potentiated noradrenaline-induced venoconstriction, an action also observed with β -adrenoceptor blockers such as propranolol (Collier *et al.*, 1972) and attributable to blockade of β -(dilator) adrenoceptors in peripheral vessels, thereby enhancing α -adrenoceptor mediated constriction.

Previous assessments of β -adrenoceptor blocking drugs in the forearm arterial bed (Brick, Glover, Hutchinson & Roddie, 1966) have been performed by determining dose-response relationships for isoprenaline before and during a continuous infusion of

the antagonist. In these circumstances, effective β -adrenoceptor blockade may be achieved with much lower concentrations of antagonist than those used in the present study. However, this method was inappropriate for the study of SK & F 92657 in which a short (2 min) infusion was chosen in order to observe the timing of onset of its dilator action. The latent period between a short infusion of 400 μ g SK & F 92657 and the appearance of arteriolar dilatation allowed us to examine the β -adrenoceptor blocking action of the drug by determining dose-response relationships for isoprenaline before and immediately after this short infusion. When these results are compared to those obtained previously in this laboratory, using a similar technique, propranolol appears to be approximately ten times more potent on a weight for

weight basis than SK & F 92657 in blocking the effects of isoprenaline on forearm blood flow. This difference in β -adrenoceptor blocking potency cannot be accounted for solely in terms of molecular weight, but is of the same order as that seen after oral administration of the two drugs (Pitcher, Curry & Trounce, 1980).

Thus the results indicate that SK & F 92657 causes arteriolar dilatation and β -adrenoceptor blockade when infused locally into the peripheral blood vessels of man. Further studies are indicated to evaluate this drug as potential antihypertensive agent.

We are grateful to the Clinical Research Department, Smith, Kline & French Research Ltd for supporting this work.

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(Received March 18, 1980)