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Sulfinpyrazone increases the number of β-adrenoceptors on intact human lymphocytes

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Sulfinpyrazone increased the number of β-adrenoceptors (+17–40%) on intact human lymphocytes at concentrations ranging from 10^{-5} to 2×10^{-4} M. It affected the B_{max} (187 ± 6 and 142 ± 5 fmol mg^{-1} protein respectively with and without sulfinpyrazone, $P < 0.001$) but did not influence K_D (4·4 ± 0·3 and 4·6 ± 0·7 nM with and without the drug). This effect was observed on either washed or unwashed lymphocyte membrane preparations. These findings have several pharmacological and therapeutic implications.

Sulfinpyrazone (Anturane) is a potent uricosuric agent also active as platelet inhibitor or antithrombotic agent (Margulies et al 1980). Recent studies suggest that it is useful in the prevention of sudden death (The Anturane Reinfarction Trial Research Group, 1980) or reinfarction (The Anturan Reinfarction Italian Study Group, 1982) in patients surviving recent myocardial infarction, though the results are controversial (Loeliger 1982). Sulfinpyrazone's antithrombotic properties do not completely account for these results. The prevention of sudden death suggests action on adrenergic heart innervation, as pointed out in a study in cats (Dix et al 1982).

The density of β-adrenoceptors in patients with heart failure was found to be reduced both on lymphocytes (Colucci et al 1981) and in the heart (Bristow et al 1982), thus explaining to some extent the loss of contractile function.

As an approach to obtaining more information about the mechanism of action of sulfinpyrazone we studied the effects of the drug on the β-adrenoceptor of intact human lymphocytes.

Materials and methods

Peripheral blood lymphocytes from 15 donors were isolated by density gradient centrifugation on Ficoll-Hypaque (Eurobio, Paris, France) according to Böyum (1968). The lymphocyte fractions were washed twice with phosphate-buffered saline (PBS) (Eurobio) and then incubated in RPMI-1640 medium supplemented with 20% foetal bovine serum (Gibco, Biocult, Glasgow, Scotland) for 45 min at 37 °C in plastic petri dishes (≠ 3003, Falcon Plastics, Oxnard, Calif.). Non-adherent cells were collected and washed with PBS. The final cell preparation contained approximately 94% lymphocytes, 5% monocytes and <2% polymorphonuclear leucocytes. Crude lymphocyte membranes were prepared by continuous sonication with a Branson sonicator for 10 s (output control 5) and were then either washed twice with PBS or not.

Binding experiments on intact lymphocytes were performed according to Watanabe et al (1981) and Krall et al (1980) with slight modifications. The standard reaction mixture (1 ml) consisted of 50 mM Tris-HCl buffer (pH 7·4), 85 mM KCl, 15 mM NaCl, 10 mM $MgCl_2$, 0·4 mM ascorbic acid, 1 mM EDTA, 10 μM pargyline and $2–4 \times 10^6$ intact lymphocytes. Incubation (15 min at 37 °C) was stopped by the addition of 4 ml of ice-chilled Tris buffer followed by rapid filtration through Whatman GF/B glass fibre filters under vacuum and three additional 4 ml washes. For saturation experiments six different concentrations of [^{3}H]dihydroalprenolol ([^{3}H]DHA) (spec. act. 51 Ci $mmol^{-1}$, NEN) were used ranging from 0·5 to 6 nM (each assayed in triplicate).

Saturation binding isotherms were analysed either by Scatchard plots (Scatchard 1949) or a non-linear regression computer program (Sacchi Landriani et al 1983). The Hill coefficient (Hill 1910) was determined. Non-specific binding was defined as [^{3}H]DHA binding in the presence of 30 μM (±)-propranolol. The amount of non-specific binding ranged from 45 to 65% of total binding, depending on the [^{3}H]DHA concentration considered.

For lymphocyte membrane binding (about 120 μg protein per sample) non-specific binding was defined as [^{3}H]DHA binding in the presence of 1 μM (−)-propranolol according to Meurs et al (1982). The remaining binding procedures were similar to those for intact cells. Amounts of protein were determined by the method of Lowry et al (1951). Drugs were obtained from the following sources: sulfinpyrazone from Ciba-Geigy, Italy and (−)-, (+)-, (±)-propranolol from ICI, UK.

Results

As shown in Fig. 1, sulfinpyrazone caused a dose-dependent increase in [^{3}H]DHA specific binding on intact lymphocytes. This effect was detectable at the concentration of 10^{-5} and reached statistical significance at 10^{-4} and 2×10^{-4} M.

Recently Meurs et al (1982) reported that the use of 30 μM (±)-propranolol to determine non-specific binding also displaced a non-stereospecific component of [^{3}H]DHA binding. As shown in Fig. 2a, studying [^{3}H]DHA binding inhibition by (+)- and (−)-propranolol we confirmed that no stereoselectivity of [^{3}H]DHA binding was evident at (+)- and (−)-

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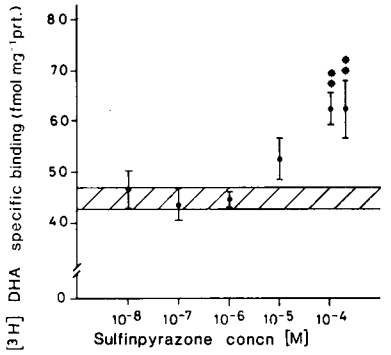

FIG. 1. Effect of sulfinpyrazone on intact human lymphocyte β-adrenoceptors. Each bar is the mean ± s.e.m. of five subjects. The shaded area shows the controls values. [^{3}H]DHA concentration = 2 nM. $^{**}P < 0.01$ Dunnett's test.

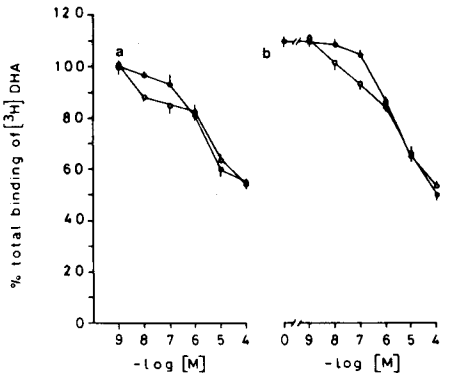

FIG. 2. Inhibition of [^{3}H]DHA binding to intact human lymphocytes by (−)-propranolol (○) and (+)-propranolol (●) in absence (a) or presence (b) of sulfinpyrazone 10^{-4} M. Each point is the mean ± s.e.m. of triplicate determinations from three to five separate experiments. [^{3}H]DHA concentration = 2 nM.

propranolol concentrations exceeding 10^{-6} M. However, in the presence of sulfinpyrazone (Fig. 2b) only the stereospecific part of [^{3}H]DHA binding was increased, the amount of [^{3}H]DHA bound in the presence of 10^{-6} M (or higher) (+)- and (−)-propranolol being unaffected by the added drug.

In saturation experiments (Fig. 3) we found that sulfinpyrazone (10^{-4} M) increased β-adrenergic receptor numbers (B_{max}) without any effect on receptor affinity (K_D). When the results were analysed by a non-linear regression computer program, they fitted well to a one-site model giving results very close to Scatchard analysis (B_{max} 183 ± 14 and 141 ± 6 fmol mg^{-1} protein, $P = 0.03$, K_D 4·1 ± 0·2 and 4·8 ± 1·0 nM respectively with and without the drug).

Hill's coefficient was (mean ± s.e.m.) 0·984 ± 0·006 and 0·985 ± 0·005 respectively in the presence and absence of sulfinpyrazone. The effect of sulfinpyrazone remained significant when binding density was calculated as fmol per mg of protein or fmol cells ×10^6. In-vitro, the effect of sulfinpyrazone on lymphocyte membranes was to significantly increase [^{3}H]DHA (2·5 nM) binding on either washed or unwashed membrane preparations [unwashed membrane (fmol ml^{-1} protein): control 31 ± 8, with drug 10^{-4} M 40 ± 9 $P <$ 0·005; washed membrane: control 20 ± 4, with drug 10^{-4} M 29 ± 5, $P < 0.02$; means ± s.e.m. of 5 separate experiments run in triplicate, Student's t-test for paired data]. The drug affected total but not non-specific binding. The solvent used for drug preparation (Na_2CO_3 2 × 10^{-7}–2 × 10^{-3} M final concentration) had no effect on [^{3}H]DHA binding (data not shown). Sulfinpyrazone had the same effect on the intact lymphocytes and membranes from 15 subjects studied.

Discussion

Intact living lymphocytes are a useful model for investigating the physiological and pharmacological regulation of β-adrenoceptors in man (Motulsky & Insel 1982). The present study showed that sulfinpyrazone

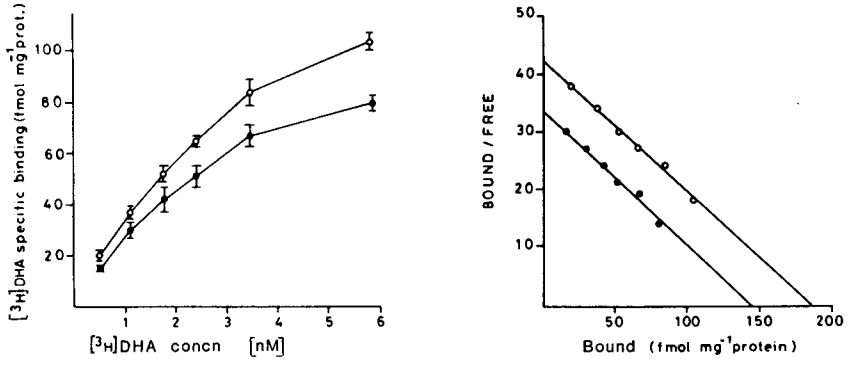

FIG. 3. Effect of sulfinpyrazone on intact human lymphocyte β-adrenoceptors in saturation experiments. On the left: saturation curves are given as mean ± s.e.m. of four subjects. On the right: Scatchard plot of the mean values. B_{max} and K_D values are mean ± s.e.m. of four subjects. ● Control K_D (nM) 4·6 ± 0·7, B_{max} (fmol mg^{-1} protein) 142 ± 5; ○ Sulfinpyrazone 10^{-4} M K_D 4.4 ± 0·3, B_{max} 187 ± 6. $^*P < 0.001$ Student's t-test.

in-vitro increased the number of β-adrenoceptors on intact human lymphocytes.

In our experimental conditions the binding of [^{3}H]DHA to intact living lymphocytes revealed a single population of sites, as indicated by the linearity of the Scatchard plot, by the good fit to the one-site model of non-linear fit and by the Hill coefficient which was very close to one. Recently Meurs et al (1982), in contrast with previous reports (Krall et al 1980; Watanabe et al 1981), suggested that the use of propranolol concentrations exceeding 10^{-6} M to define non-specific binding could inhibit low affinity non-receptor binding which was mostly evident at high [^{3}H]DHA concentrations. The fact that we found a single binding site population in saturation experiments suggests that at the [^{3}H]DHA concentrations used (0·5–6 nM)) this low affinity non-receptor binding component is not quantitatively relevant. With (−)- and (+)-propranolol as competitors the present study confirmed a previous report (Meurs et al 1982) of a non-stereospecific component of [^{3}H]DHA binding at propranolol concentrations exceeding 10^{-6} M (Fig. 2a).

However, sulfinpyraone increased only the stereospecific component of [^{3}H]DHA binding, indicating its effect is selective for stereospecific β-adrenergic binding sites. This was confirmed by the findings with lymphocyte membrane preparations where 1 μM (−)-propranolol was used to define non-specific binding. In fact sulfinpyrazone increased β-adrenoceptors on both washed and unwashed lymphocyte membrane preparations, suggesting that, cellular integrity is not necessary for this drug's effect. It has been proposed (Motulsky & Insel, 1982) that the lymphocyte membrane preparation procedure washes away some factors usually present inside the cells (e.g. ions and nucleotides), that could regulate receptor function. The fact that sulfinpyrazone was active on either washed or unwashed membrane preparations suggests the effect on membrane is independent of either cellular integrity or the presence of intracellular factors that physiologically regulate receptor functions. However the exact mechanism is as yet unclear. This drug has no direct affinity for the β-adrenoceptor, as revealed by the lack of inhibition of [^{3}H]DHA binding to its specific receptor sites, so it probably acts indirectly. Since the numbers of β-adrenoceptors in different tissues are closely related to membrane fluidity changes (Shinitzky 1983), sulfinpyrazone may increase [^{3}H]DHA binding sites by changing membrane fluidity.

It is interesting to note that this effect was observed at drug concentrations (10^{-5}–2×10^{-4} M) very close to the plasma concentrations ($2–4 \times 10^{-5}$ M) (Pedersen & Jakobsen 1981) attained after therapeutic drug doses. It thus appears that sulfinpyrazone affects adrenergic nerve terminal transmission by acting on specific adrenergic receptors. This could be of interest in therapy for its effect on adrenergic heart regulation. The drug's antiarrhythmic efficacy (Margulies et al 1980) or the prevention of sudden death (The Anturane Reinfarction Trial Research Group, 1980) may be unrelated to its antithrombotic properties and the present findings suggest an adrenergic-related mechanism of action, but this hypothesis is still purely speculative.

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