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Effects of Atenolol, Labetalol and Methyldopa on Endogenous Antioxidants In-vitro

G. CHEN§, R. WILSON, G. CUMMING*, W. E. SMITH†, W. D. FRASER‡, J. J. WALKER* AND J. H. MCKILLOP

*University of Glasgow, Department of Medicine, *Department of Obstetrics, Royal Infirmary, 10 Alexandra Parade, Glasgow G31 2ER, †Department of Chemistry, University of Strathclyde, Glasgow G1 1XQ, and ‡Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool L69 3BX, UK*

Abstract

The aim of this study was to investigate whether atenolol, labetalol and methyldopa, which are commonly used for the treatment of hypertension in pregnancy, can induce antioxidant activity.

Reactive oxygen species scavengers (plasma thiol, red cell lysate thiol, red cell superoxide dismutase, red cell membrane thiol and plasma glutathione) were measured after incubation of peripheral blood with atenolol and methyldopa, respectively.

The results showed that atenolol and labetalol could significantly raise the levels of plasma thiol and membrane thiol but had no effects on lysate thiol, superoxide dismutase and glutathione. The effects on membrane thiol occurred after 60 min incubation and on plasma thiol after 120 min incubation.

The data also suggest that atenolol and labetalol at lower concentrations tend to have additive effects on reactive oxygen species scavengers but at higher concentrations do not.

Methyldopa had no significant effect on any of the parameters measured.

These findings suggest that atenolol and labetalol are able to induce higher levels of antioxidant activity.

Atenolol, labetalol and methyldopa are antihypertensive drugs, which are commonly used for the treatment of hypertension, angina and arrhythmia. Atenolol and labetalol are β-blockers. Methyldopa acts as a stimulator of α_2-adrenergic receptors. Published literature has indicated that β-blockers are able to provide significant protection against reactive oxygen species (ROS)-mediated sarcolemmal membrane lipid peroxidation (Mak & Weglicki 1988). They act as simple, reversible xanthine oxidase (an important enzymatic superoxide radical source) inhibitors (Janero et al 1989). To date the literature regarding the relation between β-blockers and endogenous antioxidants in peripheral blood is limited. Likewise, relatively few studies have considered the effects of methyldopa on ROS or endogenous antioxidants in peripheral blood. ROS have been implicated in cardiac diseases (Krzanowski 1991; Wisdom et al 1991). Therefore, from a therapeutic standpoint, it would be of value to determine if antihypertensive drugs have favourable effects on ROS scavengers. In this paper, we have determined in-vitro the effects of atenolol, labetalol and methyldopa on the following antioxidant markers: red cell superoxide dismutase (SOD), plasma thiol (PSH), red cell lysate thiol (LSH), red cell membrane thiol (MSH) and plasma total gluthathione (GSH).

Materials and Methods

Reagents and supplies

5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), glutathione, glutathione reductase (type III), *o*-dianisidine, riboflavin and superoxide dismutase were purchased from Sigma Chemicals (Poole). 5-Sulphosalicylic acid (SSA) was from BDH Chemical Ltd, Poole, UK. Atenolol was supplied by ICI Pharmaceuticals, Macclesfield, UK, labetalol by Glaxo (Middlesex) and methyldopa by Merck Sharp and Dohme (Essex).

Peripheral blood and drug concentrations

Heparinized peripheral blood was taken at 10 00 to 11 00 h from 14 healthy normal subjects who had no family history of hypertension or autoimmune diseases and had a normal diet. Peripheral blood was incubated with atenolol, labetalol and methyldopa respectively for 15–120 min at 37°C.

SOD, PSH and LSH assay

The methods for SOD, PSH and LSH have been described elsewhere (Banford et al 1982a, b). Briefly, SOD activity was measured according to the method of Misra & Fridovich (1977) based on the increase in the rate of photo-oxidation of *o*-dianisidine. Intra and inter-assay variations in our laboratory were 4·6 (n = 13) and 7·0% (n = 13). PSH and LSH levels were measured using the thiol-disulphide interchange reaction between DTNB and biological thiols (Ellman 1959). The intra- and inter-assay variations for PSH assays were 1·2 (n = 10) and 1·7% (n = 10), respectively, and for LSH 1·0 (n = 10) and 7·0% (n = 10).

Measurement of MSH (Reglinski et al 1988)

This assay is based on the reaction of MSH with Ellman's reagent (DTNB). Ellman's reagent can be used to induce an oxidative stimulus on the exofacial membrane sulphydryl group of the human red cell, thus making it a chemical probe of the sulphydryl population.

§Present address and correspondence: G. Chen, Department of Paediatrics, The Rayne Institute, University College London Medical School, 5 University Street, London WC1E 6JJ, UK.

$$ESSE + MSH \rightarrow ES^- + MSSE + H^+$$

Red cells were washed three times with PBS and were incubated with 1×10^{-4} M Ellman's solution at 37°C for 30 min. The suspensions were centrifuged at 3000 rev min^{-1} for 10 min and the supernatant obtained. The spectrum of the supernatant was recorded at 600–350 nm. Absorbance values were taken at wavelengths of 412 and 541 nm, respectively. The absorbance value obtained at 541 nm was to ensure that cell lysis could be monitored. The sample was discarded if cell lysis occurred. The number of thiols per cell was calculated from the molar extraction coefficient (13 600) and Avogadro's number.

Measurement of GSH

The GSH content of the plasma was measured enzymatically at 25°C by the method of Anderson (Greenwald 1985). Plasma was separated by centrifugation at 12 500 *g* for 2 min and the plasma was immediately deproteinized by adding 0·5 vol 10% (w/v) SSA. The reaction medium contained 143 mmol L^{-1} sodium phosphate (pH 7·5), 6·3 mmol L^{-1} EDTA, 6 mmol L^{-1} DTNB and 2 units mL^{-1} glutathione reductase. A 10-μL sample was added to the reaction mixture (final volume 1·0 mL) and the initial rate of reduction of DTNB determined spectrophotometrically at 412 nm. With each assay a standard curve was generated with known amounts of reduced GSH.

cAMP (3'5'-cyclic adenosine monophosphate) assay

After peripheral blood was treated with atenolol, labetalol or methyldopa as stated above, plasma was separated and kept at −70°C until assay. cAMP was measured using an in-house radioimmunoassay after acetylation of the samples with triethylamine/acetic anhydride (2 : 1). The cAMP antisera has been described previously (O'Reilly et al 1986). The assay has a detection limit of 30 pmol L^{-1} and a between-batch coefficient of variation of 10–15% over the working range of the assay.

All samples were assayed in duplicate for various antioxidant measurements and cAMP determination.

Statistics

Data are expressed as mean ± s.d. and analysed for differences by partition of the sums of squares. A P value of 0·05 or less was considered to be significant.

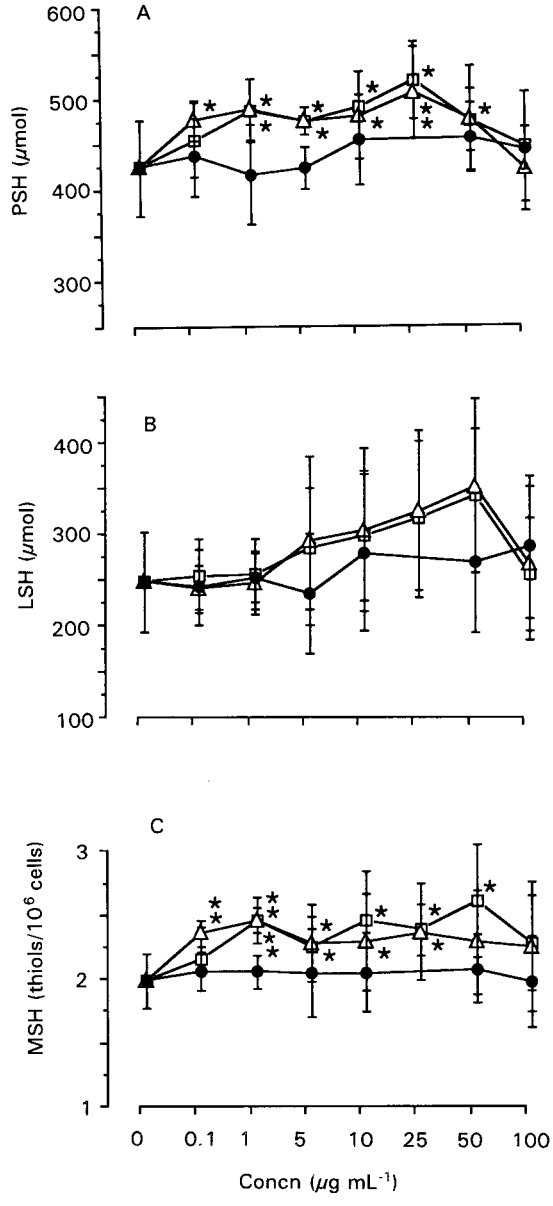

FIG. 1. The effects of atenolol (□), labetalol (△) and methyldopa (●) on PSH, LSH and MSH. Free radical scavengers were measured after 120 min incubation of each drug with peripheral blood. Data are expressed as mean ± s.d. $*P < 0.05$, $**P < 0.01$.

Results

Atenolol and labetalol significantly affected PSH and MSH levels, but had no effect on LSH (detailed data are not presented). Atenolol at concentrations from 1 to 50 μg mL^{-1} and labetalol at concentrations from 0·1 to 25 μg mL^{-1} increased PSH levels significantly (Fig. 1A). The dose–response curves were bell shaped with maximum effects being detected at a concentration of 25 μg mL^{-1} for both atenolol and labetalol. Although Fig. 1B shows that both atenolol and labetalol at several concentrations raised the level of LSH, there were no significant differences. Atenolol at concentrations of 1–50 μg mL^{-1} and labetalol at concentrations of 0·1–25 μg mL^{-1} had significant effects on MSH (Fig. 1C). Atenolol and labetalol had no significant effect on SOD activity and GSH content. Methyldopa had no significant effect on any of the parameters measured.

The results of a time-course study are shown in Fig. 2. Atenolol (25 μg mL^{-1}) and labetalol (25 μg mL^{-1}) were incubated with peripheral blood and the levels of various antioxidant markers were measured after 15, 30, 60 and 120 min incubation. This study indicated that atenolol and labetalol exerted significant effects on PSH after 120 min incubation with peripheral blood, and on MSH after 60 min incubation.

Solutions of atenolol, labetalol and methyldopa at different concentrations were determined for PSH, LSH, SOD, MSH and GSH activity to find out whether the chemical forms of these physiological ROS scavengers exist in the drugs. The levels of various scavenging agents

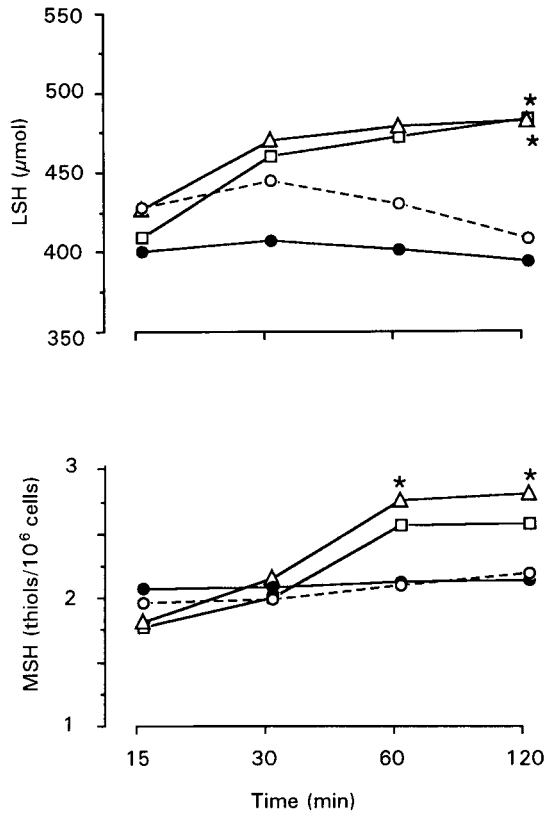

FIG. 2. The activity of free radical scavengers after incubation of peripheral blood with atenolol (□, 25 μg mL^{-1}), labetalol (△, 25 μg mL^{-1}) and methyldopa (●, 25 μg mL^{-1}), respectively at different times. ○ Control. *$P < 0.05$.

in drug solutions were not significantly different from those in phosphate-buffered saline solution alone (detailed results are not presented).

Additive effects of atenolol and labetalol on ROS scavengers were observed at 5 μg mL^{-1} atenolol plus 5 μg mL^{-1} labetalol; showing a similar effect to those observed at 10 μg mL^{-1} atenolol or 10 μg mL^{-1} labetalol ($P > 0.05$), and were more effective than 5 μg mL^{-1} atenolol or 5 μg mL^{-1} labetalol alone (Table 1). However, 50 μg mL^{-1} atenolol plus 50 μg mL^{-1} labetalol showed no such additive effects.

Following incubation of whole blood with atenolol, labetalol and methyldopa respectively, no significant differences in plasma cAMP levels were found (detailed results are not presented).

Discussion

In this study, we have shown that when incubated with whole blood, atenolol and labetalol at certain concentrations are able to raise the levels of the ROS scavengers PSH and MSH, suggesting that they possess some antioxidant properties. Our study has excluded the possibility that atenolol and labetalol contain chemical forms of ROS scavengers.

Both atenolol and labetalol are β-blockers, while methyldopa, which did not show any antioxidant effects in these experiments, is not. On this basis, it can be inferred that the

Table 1. Effects of atenolol and labetolol alone or in combination on PSH and MSH.

Drug added		PSH	MSH
Atenolol (μg mL^{-1})	Labetalol (μg mL^{-1})	(μmol L^{-1})	(10^6 cells)
—	—	417 ± 53	200 ± 25
5	—	455 ± 40	224 ± 21
10	—	482 ± 40	247 ± 40
50	—	479 ± 31	229 ± 17
—	5	448 ± 38	232 ± 39
—	10	473 ± 48	236 ± 39
—	50	458 ± 41	255 ± 25
5	5	506 ± 21	254 ± 39
50	50	472 ± 34	252 ± 30

n = 6.

antioxidant effects of β-blockers may occur by a mechanism involving the β-receptor.

Since one of the major pharmacologic mechanisms of β-adrenoceptor blockers is to decrease intracellular cAMP through the inactivation of adenosine cyclase (Lefkowitz et al 1984), it could be postulated that the antioxidant effects of β-blockers observed in this study are due to decreased cAMP concentrations. Furthermore, elevation of cAMP levels has been suggested to increase ROS production in other studies (Meltzer et al 1989; Yukawa et al 1989). Therefore, it is possible that by reducing ROS formation, β-blockers may take the place of the physiological ROS scavengers and thus cause a rise in levels of PSH and MSH. In this study, we found that plasma cAMP level was not significantly different among the three drugs used. This excludes the possibility that the changes found in PSH and MSH levels were mediated by cAMP.

To avoid possible cell activation consequent to the purification procedure (Shappel et al 1990), we did not purify any blood components. By incubating the drugs with whole blood, we studied the effects of β-blockers on ROS. It has been documented that blood cells such as lymphocytes not only produce ROS but also modulate ROS production via cytokines, including interferon-γ and interleukin-2 (Salisbury & Calhoun 1990; Klausner et al 1991). Therefore, it is also possible that the β-blockers reduce ROS activity by altering cytokine production or release. However, the time course of blood cell activation to produce cytokines is measured in hours or days. In the present experiment, the significant effects of β-blockers were detectable as early as 60 min after the beginning of incubation. Thus, any change in cytokines, caused by the β-blockers, is unlikely to be the main mechanism by which they exert their antioxidant effects.

It has been reported that following therapeutic doses of the drugs the concentration of atenolol in plasma ranges from 0·3 to 2·2 μg mL^{-1} and the concentration of labetalol from 0·093 to 0·271 μg mL^{-1} (McNeil et al 1979; Cruickshank 1980). Therefore, the clinical concentrations of both agents appear to be the concentrations at which the antioxidant activity was found in this study. Antioxidant effects were also observed at concentrations well above the range needed clinically to block β-adrenergic receptors. The degree of antioxidant effect did not always increase with

drug concentration and at concentrations $<100\,\mu g\,mL^{-1}$ no effects were observed. Furthermore, an additive effect of β-blockers occurred at lower doses and not at higher doses. These findings suggest that the effects observed are contributed by an interaction of drugs with specific membrane receptors, probably β-receptors.

Mak and associates (Mak & Weglicki 1988; Mak et al 1989) have demonstrated that β-blockers can interact with the membrane hydrophobic components and subsequently inhibit ROS propagation in the membrane. Concomitantly, they protect the membranes against ROS-induced lipid peroxidation. Janero et al (1989) have shown that propranolol (a β-blocker) can suppress superoxide generation by inhibiting xanthine oxidase. Our study found that atenolol and labetalol can exert antioxidant activity by raising the levels of ROS scavengers such as PSH and MSH. Although the mechanism responsible for the observed effects of β-blockers on ROS scavengers remains to be elucidated, the potential therapeutic significance of these drugs has been deemed to be of scientific interest.

Jones et al (1981) have demonstrated that methyldopa stimulates the conversion of the primary catalase peroxide complex (the active form of the enzyme) to the catalytically inactive secondary catalase peroxide complex, leading them to conclude that methyldopa may initiate an ROS process. Using ROS scavengers as indirect indexes of ROS activity, our study showed that methyldopa has no significant effects on ROS, producing neither augmentation nor reduction of ROS levels.

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