The relationship between some beta-adrenergic mediated responses and plasma concentrations of adrenaline and cyclic AMP in man

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Abstract To test the hypothesis that increments in plasma cyclic AMP during beta-adrenergic stimulation reflect integrated second messenger function of the tissues activated by the agonist, graded adrenaline infusion resulting in plasma adrenaline concentrations within the physiological range was performed in 8 healthy subjects with and without concomitant beta-adrenoceptor blockade by iv propranolol. A significant correlation was found between increments in plasma adrenaline and plasma cyclic AMP in the experiments without beta-blockade; during concomitant beta-blockade the increase in plasma cyclic AMP concentrations at low adrenaline infusion rates was prevented, whereas a small increase in cyclic AMP was found at high adrenaline infusion rates, probably owing to incomplete beta-receptor blockade. Likewise, the adrenaline-induced increments in blood substrates (glucose, lactate, glycerol and betahydroxy butyric acid) were significantly reduced but not completely prevented by beta-blockade. We conclude that an altered relationship between beta-agonist concentrations and plasma cyclic AMP may provide evidence for the existence of differences in beta-adrenergic sensitivity in man.

Whereas the sources of basal concentrations of cyclic AMP (cAMP) in plasma in man may be manyfold, the increase in plasma cAMP upon beta-adrenergic stimulation (e.g. by adrenaline) is probably due to leakage of cAMP into the blood following activation of the beta-receptor (1,2). Thus, increments in plasma cAMP after beta-agonist stimulation may reflect integrated second messenger function of the tissues activated by the agonist. In experimental situations where changes in beta-adrenergic sensitivity are likely to exist, an altered relationship between plasma concentrations of beta-adrenoceptor agonist and plasma cAMP might therefore support the existence of such differences. This in vivo method for the determination of beta-adrenergic sensitivity is based on the assumption that beta-adrenoceptor blockade during concomitant beta-agonist stimulation prevents the increase in plasma cAMP. However, such data are not available.

The aim of the present study was therefore to study the relationship between plasma concentrations of catecholamines and cAMP during graded infusions of adrenaline and to compare these with changes in physiological variables induced by the infusion. Finally, the effect of beta-adrenergic blockade during these experimental conditions was assessed.

Subjects and Methods

Study population
Eight healthy male subjects, mean age 34 years (range 27-42) volunteered for the study after giving informed consent. The subjects took no drugs. The experiments were approved by the local Ethical Committee.

Experimental protocol
All experiments started at 08.00 h, the subjects having fasted and abstained from tobacco and alcohol overnight. The subjects were studied two times in randomized order. On one occasion an iv cannula was inserted into a cubital vein in both arms, whereupon the subjects rested
supine for 15 min. Subsequently blood samples were drawn two times at 15-min interval for the determination of preinfluence concentrations of hormones and metabolites, and blood pressure (sphygmomanometer and cuff) and heart rate (auscultation) were recorded. Immediately after the completion of basal recordings, a graded iv infusion of adrenaline was begun at a constant infusion rate of 0.5 μg/min for 1 h, 2.5 μg/min for 1 h, and 5 μg/min for 1 h. Blood samples for the determination of cAMP, adrenaline, noradrenaline, insulin, glucagon, glucose, lactate, glyceral, and beta hydroxy butyric acid were drawn at the end of each infusion period, and heart rate and blood pressure were recorded. On the other occasion an identical experiment was performed with the exception that 5 mg propranolol was given iv concomitantly with the beginning of the adrenaline infusion, followed by an infusion of propranolol at a rate of 80 μg/min throughout the rest of the experiment.

Finally, noradrenaline was infused at 5 μg/min for 1 h in two healthy male subjects. Blood samples for the determination of catecholamines and cAMP were drawn before and at the end of the infusion period.

cAMP assay

Extraction of cAMP from blood. Five ml blood was taken into glass tubes containing 100 μl sodium EDTA solution (100 g/l). Plasma was separated and stored at −20°C until analysis.

Plasma, 0.5 ml, was mixed with 0.5 ml saline and 40 μl [3H]-cAMP (New England Nuclear, NET 275, 33 Ci/ml diluted 1:4000); 2 ml acid ethanol was added and the solution was centrifuged at 1500 × G for 5 min. The supernatant was transferred to glass tubes and evaporated to dryness under nitrogen at 50°C. The dry residue was dissolved in 4 ml of buffer and washed with 1 ml petroleum ether (bp. 40°C–60°C). The organic phase and upper layer of the lower phase was carefully removed by suction and 3 ml (% of the total water phase) was loaded onto Sep-Pak C18 cartridges (Waters, USA). The cartridges were washed with 5 ml RIA buffer and the cAMP eluted with 2 ml of methanol. Finally, the methanol was evaporated under nitrogen at 50°C and the residue dissolved in 1 ml RIA buffer. Of this solution 100 μl was removed for liquid scintillation counting and recovery estimation, and various aliquots (25, 50 and 100 μl) were subjected to radioimmunoassay.

Tracer for radioimmunoassay. Succinyl-cAMP tyrosine methylester, Sc-AMP-TME (Sigma M 2257) was used for iodination by a iodogen procedure. A plastic tube was coated with iodogen (1,3,4,6 tetrachloro-3a, 6a-diphenyl Glycoluril, Pierce, UK) using 150 μl of a solution containing 1 mg iodogen/25 ml dichloromethane evaporated to dryness; 15 μl of Sc-AMP-TME (1 g/l water), 80 μl 0.5 mol/l sodium phosphate buffer, pH 7.4, and 100 μl Na125I (600 μCi) were transferred to the iodogen tube. The solution was vortexed and the iodination allowed to proceed for 2 min at room temperature. The total reaction mixture was loaded onto a Sephadex G25 column (1 × 75 cm) with 1000 μl of water. Gel filtration was carried out using 0.1 mol/l phosphate buffer, pH 7.4, as eluant and fractions of 3 ml were collected. Only one radioactive peak appeared, with a peak at 123 ml. Selected peak fractions of 3 ml were frozen at −20°C after addition of 125 μl of 2% human albumin. The tracer solution could be used at least 6 months in the radioimmunoassay.

Immediately before use in radioimmunoassay the tracer was diluted 1:200 in RIA buffer giving a count rate of 7000 cpm per 100 μl with recent preparations of the tracer.

Radioimmunoassay. Standards of cAMP (Sigma A 4137) covering a range from 0.39 to 50 nmol/l buffer were prepared from a stock solution containing 1 mmol cAMP per litre. The pH 6.2 buffer used for RIA was prepared from 60 ml 1 mol/l NaOH, 2 g Titrilplex® III, 4 ml of a 5% NaN₃ solution, 0.05 mol/l acetic acid, 935 ml cAMP antiserum was obtained from Chemicon International Inc (anti-rabbit lot 493-5). One ampoule (2000 tubes) was dissolved in 2 ml of RIA buffer and 25 μl of a dilution (1:25) was used in the RIA procedure. Cross-reactions with ATP, ADP and AMP were less than 0.0001%.

Incubation for radioimmunoassay was performed using 100 μl 125I-Sc-AMP TME, 25 μl diluted antiserum, 100 μl standard solution or plasma extract, and 200 μl RIA buffer. After incubation for 72 h at 4°C, 200 μl of a solution of solid-phase second antibody-coated cellulose suspension (Sac-cel, RD 70, Wellcome Diagnostics, UK) was added. The tubes were rotated for 3 h at room temperature and centrifuged at 2000 × G for 5 min. The supernatant was decanted and counting of the remaining bound radioactive fraction was carried out in a gamma counter; 10 000 counts were accumulated in all cases. The final results were corrected for radioactive recovery.

The radioimmunoassay procedure was characterized by analysis of duplicate samples throughout the study. Consistently, a coefficient of variation of 2.4% was observed. The standard curve demonstrated an ID₅₀ of 0.50 pmol/tube (i.e. the amount of cAMP required to decrease the percentage of antibody bound tracer from 100 to 50% of initial binding); ID₅₀ was 0.059 pmol/tube. Within- and between-assay variations were evaluated using 5 plasma pools. Within-assay variation was estimated on pool A: 22 ± 2.55 nmol/l plasma (mean ± SD, N = 10, CV = 11.3%), pool B: 18.2 ± 2.78 nmol/l plasma (N = 5, CV = 15.3%) and pool C: 16.0 ± 3.58 nmol/l plasma (N = 6, CV = 22.5%). Between-assay variation across the study was 13.7% for pool D: 17.8 ± 2.44 nmol/l, and 17.2% for pool E: 17.3 ± 2.98 nmol/l plasma.

Other hormones

Plasma adrenaline and noradrenaline were measured by a radioenzymatic technique previously described (3). Insulin was measured by radioimmunoassay (4). Pancreatic
glucagon (using antibody 4305) and gut glucagon (using antibody 4304) were measured with previously described radioimmunoassays (4).

Substrates
Plasma glucose, lactate, glycerol, and beta-hydroxy butyric acid were measured with enzymatic methods as previously described (5).

Statistical analysis
The data in the text and figures are given as means ± SEM. Statistical significance was determined by analysis of variance corrected for repeated measurements (6) as well as by the t-tests for paired and unpaired comparisons and linear regression analysis. A p-value (two-tailed) less than 0.05 was considered significant.

Results
Plasma adrenaline increased with increasing adrenaline infusion rate in both experiments (adrenaline infusion and adrenaline plus propranolol infusion) (Fig. 1). The increase in plasma adrenaline was significantly greater (p<0.05) in the propranolol infusion experiment than during adrenaline infusion. Plasma noradrenaline increased insignificantly during adrenaline infusion, whereas plasma noradrenaline decreased significantly (p<0.025) during combined adrenaline and propranolol infusion (Fig. 1).

Plasma cAMP increased significantly during adrenaline infusion (Fig. 1); during combined adrenaline and propranolol infusion there was a small but significant increase in plasma cAMP at the end of the experiment (Fig. 1). A significant correlation (p<0.025) was found between increments in plasma adrenaline and plasma cAMP during adrenaline infusion (Fig. 2); no significant correlation was found during adrenaline and propranolol infusion.

Plasma insulin increased significantly (p<0.01)
during adrenaline infusion (Fig. 1), whereas a small but significant decrease was found during combined adrenaline and propranolol infusion (p<0.05).

No significant changes were found in plasma concentrations of pancreatic glucagon (as measured by antibody AB 4305) and gut glucagon (AB 4304) in any of the experiments (Fig. 1). Blood glucose increased significantly in both infusion experiments (Fig. 3); at the end of the experiments, the increase in blood glucose was significantly greater in the adrenaline infusion experiments than with the combined adrenaline and propranolol infusion (p<0.05).

Similarly, lactate, glycerol and betahydroxy butyric acid concentrations increased significantly at adrenaline infusion rates 2.5 μg/min and 5 μg/min (Fig. 3); smaller but still significant increments were found at 5 μg/min in the combined infusions (p<0.05).

Heart rate increased significantly at 2.5 μg/min and at 5 μg/min during adrenaline infusion (Fig. 4); a significant decrease was found in heart rate at 5 μg/min in the combined infusion experiment (Fig. 4).

Systolic blood pressure increased and diastolic blood pressure decreased during adrenaline infusion; mean blood pressure (calculated as diastolic pressure + ½ (systolic – diastolic pressure)) did not change significantly during these experimental conditions. During combined adrenaline and propranolol infusion there was a significant increase in both systolic and diastolic blood pressure, and mean blood pressure increased significantly (p<0.025) (Fig. 4).

Plasma cAMP decreased slightly (from 20 to 19 nmol/l) after noradrenaline infusion at 5 μg/min for 1 h; plasma noradrenaline increased from 1.61 to 4.79 nmol/l in these experiments.

Discussion

The main finding of the present study is a linear relationship between increments in plasma adrenaline and plasma cAMP concentrations during graded adrenaline infusions in healthy subjects. The increase in plasma cAMP was abolished by concomitant infusion of a beta-adrenoceptor blocking agent at low adrenaline infusion rates (0.5 and 2.5 μg/min), whereas a small but significant increment was found at an adrenaline infusion rate of 5 μg/min, probably owing to incomplete beta-adrenoceptor blockade. Thus, measurements of plasma cAMP during beta-agonist stimulation may be employed in the assessment of whole-body beta-adrenoceptor responsiveness in man.

In vitro stimulation of intact cells (e.g. lymphocytes) with adrenaline results in increased cAMP production which is blocked by addition of propranolol to the medium (7,8). Our results suggest that this mechanism is also operative in the intact organism stimulated with adrenaline, resulting in plasma concentrations within the physiological range. Furthermore, the relationship between increments in plasma adrenaline and the derived increments in cAMP production is linear, a finding which is in agreement with results from in vitro studies (7,8). The present data seem to indicate that deviations from the relationship between plasma concentrations of adrenaline and cAMP in healthy man suggest altered beta-adrenoceptor function and/or changes in adenylate cyclase activity in the intact organism, as previously proposed (9-11). Evidently, deductions as to which tissues involved are not possible from such data.

The increase in plasma cAMP observed at adrenaline infusion at 5 μg/min during beta-adrenoceptor blockade may be due to increased adenylate cyclase activity owing to stimulation of target cells by peptides released by adrenaline, or to incomplete beta-adrenoceptor blockade. Although the former alternative cannot be ruled out, it should be emphasized that glucagon, which after binding to its receptor does activate adenylate cyclase (12), did
not change during these experimental conditions. Therefore, the increase observed in plasma cAMP is not attributable to glucagon release. The latter alternative (i.e., incompleteness of beta-adrenoceptor blockade) seems more likely, since beta-adrenergic responses (i.e., blood glucose, lactate, glycerol, and betahydroxy butyric acid) were not entirely abolished by the blockade. It should be noted that the early increments in these parameters (i.e., adrenaline infusion rate of 2.5 μg/min) is probably at least in part due to the suppression of plasma insulin observed during concomitant beta-adrenoceptor blockade, a finding which is explained by alpha-adrenergic inhibition of insulin release (13). The alpha-adrenergic stimulating property of adrenaline is not a candidate for the increase in plasma cAMP, since alpha-adrenoceptor stimulation does not increase adenylate cyclase activity (14), as confirmed by lack of increase in plasma cAMP during iv noradrenaline infusion in the high physiological range in the present study.

The blood pressure response to adrenaline was

Fig. 3.
Blood concentrations of glucose, lactate, glycerol and betahydroxy butyric acid (BoH) before and during graded adrenaline infusion with (— —) and without (——) concomitant beta-adrenoceptor blockade. For details, see legend to Fig. 1.

Fig. 4.
Heart rate and blood pressure during graded adrenaline infusion with (— —) and without (——) concomitant beta-adrenoceptor blockade. For details, see legend to Fig. 1.
converted to a pressor response characteristic of alpha-adrenoceptor stimulation by concomitant beta-adrenoceptor blockade, the beta-blockade unmasking the alpha-adrenergic component of adrenaline. This mechanism is likely to be responsible for hypertensive crisis observed during hypoglycemia in diabetic patients treated with beta-adrenoceptor blockers (15,16).

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References


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