EFFECT OF TRAPIDIL ON PROSTACYCLINE FORMATION BY RABBIT HEART TISSUE

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A labile substance with vasodilator and antiaggregating activity is formed in the vascular wall. It has been shown that this substance is prostacycline (PGI_2), a member of the family of prostaglandins (PG) [6]. Disturbance of PGI_2 synthesis, leading to initiation of thrombus formation and constriction of the lumen of the vessels may be one cause of cardiovascular diseases [1, 6, 7]. It was therefore decided to obtain and study preparations increasing the formation of endogenous PGI_2.

Trapidil [a derivative of triazolo(1,5-a)pyrimidine] was synthesized in 1971 [13]. It was subsequently shown to have a vasodilator and antiaggregating effect, and also to increase PGI_2 formation by a strip of rat aorta [5, 10, 12]. However, different vessels react to different degrees to administration of this compound and the decrease in vascular resistance differs in different parts of the vascular system [9]. The primary action of trapidil (like that of exogenous PGI_2) is to produce coronary vasodilatation [12], which, it can be postulated, is due to increased formation of endogenous PGI_2 in the heart.

The object of this investigation was to study the effect of trapidil on PGI_2 formation by pieces of heart tissue, using the method of inhibition of platelet aggregation.

EXPERIMENTAL METHOD

Male chinchilla rabbits weighing 2.5-3.0 kg were anesthetized with sodium barbiturate (50-60 mg/kg body weight, intravenously). Biopsy specimens were obtained under open chest conditions during artificial ventilation of the lungs. Pieces of tissue from the left ventricle of the rabbits (100-150 mg) were quickly rinsed in physiological saline, transferred into cold 50 mM Tris-buffer (pH 8.0), and used in the experiments within 20 min of being obtained.

Blood was taken from the heart of superficially anesthetized male rabbits (sodium barbiturate, 20 mg/kg, intravenously), mixed with 3.8% sodium citrate solution (9:1), and subjected to differential centrifugation in order to obtain platelet-deprived and platelet-enriched plasma. Platelet-enriched plasma (PEP) containing 6×10^5 platelets/μl was used. ADP (from Sigma, USA) was used as the aggregating agent. Platelet aggregation was measured by Born's method [4] on the "Chrono-Log" aggregometer (England).

Pieces of heart tissue were incubated for 10 min at 37°C in 0.5 ml of 50 mM Tris-buffer (pH 8.0), containing arachidonic acid (6 μM), and saturated with oxygen. Trapidil, generously provided by Dr. Förster (Institute of Pharmacology and Toxicology, Martin Luther University, East Germany), was added to one of the tubes 1 min before the beginning of incubation. Aliquots of the incubation medium (0.03 ml) were introduced into the cuvette of the aggregometer with 0.45 ml PEP 1 min before addition of ADP (4-6 μM). The effect was expressed as percentages of inhibition of aggregation. Endogenous PGI_2 formation was assessed by comparison with the inhibitory effect of synthetic PGI_2 (from Upjohn, USA). The typical picture of the change in optical density of the plasma on addition of synthetic PGI_2 to the aggregometer cuvette is shown in Fig. 1. The results of quantitative assay of PGI_2 biosynthesis were expressed in units of determination of PGI_2-like activity during incubation for 10 min, in ng/mg wet weight of tissue. Parallel determinations of PGI and $PGF_{2\alpha}$ in the incubation medium were made by radioimmunoassay, as described previously [2].

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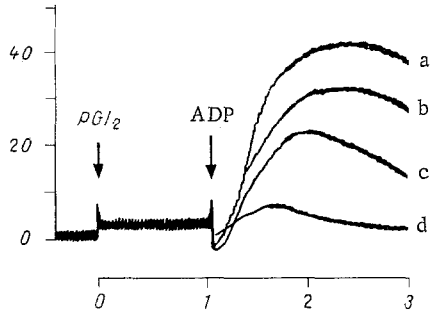

Fig. 1

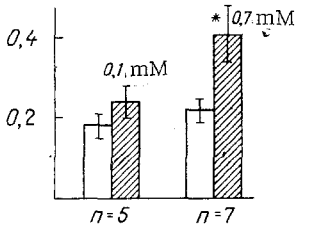

Fig. 2

Fig. 1. Changes in optical density of rabbit plasma (ΔT) containing different concentrations of synthetic PGI_2. Abscissa, time (in min); ordinate, optical density of plasma (in %). a) Control; b) dose of synthetic PGI_2 3 mg/ml; c) 6 mg/ml; d) 12 mg/ml; 10 µl of PGI_2 solution in 1 M Tris-buffer (pH 10.0) was added to 0.45 ml PEP 1 min before addition of ADP (4 µM).

Fig. 2. Increased PGI_2 formation by pieces of heart tissue under the influence of trapidil (M ± m). Ordinate, PGI_2 formation (in ng/mg tissue/10 min). Unshaded columns — control; shaded — trapidil. Final concentration of trapidil in incubation medium 0.1 and 0.7 mM. n) Number of data compared. Asterisk indicates significant increase in PGI_2 formation ($P < 0.01$).

EXPERIMENTAL RESULTS

The experimental results are shown in Fig. 2. Addition of trapidil to the incubation medium led to an increase in PGI_2 synthesis from arachidonic acid. Maximal PGI_2 formation toward the end of the 10th minute of incubation was observed with trapidil in a concentration of 0.7 mM. Trapidil can inhibit platelet aggregation [10], and for that reason the effect of trapidil itself on platelet aggregation was subjected to strict control; 10% and 20% inhibition of platelet aggregation was observed with trapidil in a final concentration of 100 and 200 µM, respectively, in the aggregometer cuvette. Practically no antiaggregating activity was observed with concentrations below 64 µM. Under these experimental conditions, the trapidil concentration in the aggregometer cuvette did not exceed 47 µM. The greater inhibition of platelet aggregation by incubation of the heart tissue with trapidil was thus due primarily to the formation of endogenous PGI_2 and not to any antiaggregating properties of the trapidil. This conclusion is confirmed by the almost complete disappearance of the antiaggregating effect of the incubation medium (both with and without trapidil) after preliminary incubation with indomethacin (0.14 mM). According to data in the literature [10], a significant increase in PGI_2 formation by strips of aorta takes place with trapidil in a concentration causing more than 50% inhibition of platelet aggregation. Under the experimental conditions used, a marked effect on PGI_2 formation by pieces of heart tissue was observed with doses of trapidil slightly smaller than those needed to exhibit its antiaggregating activity. This was evidently connected with the greater specificity of trapidil with respect to the heart. It can be tentatively suggested that not only the vascular tissue, but also the muscular tissue of the heart responds to trapidil. For instance, whereas until recently the view has been held that the PGI_2-synthesizing of the heart is concentrated mainly in the endothelium of the coronary arteries [14], evidence has now been obtained that PGI_2 is formed in the myocytes [3]. The positive inotropic and chronotropic action of trapidil on heart muscle can also be explained by increased PGI_2 formation in the myocardium [8].

On addition of trapidil to the incubation medium, incidentally, no increase was observed in the PGE and $PGF_{2\alpha}$ concentrations compared with the control.

An inhibitory effect of trapidil both on the formation of thromboxane A_2 (TXA2) in platelets and on the manifestation of its action [11] has been demonstrated in the literature. PGI_2 and TXA_2 possess directly opposite actions. Whereas one inhibits platelet aggregation and dilates the coronary vessels, the other induces thrombus formation and spasm of the coronary vessels. The ratio between the degree of their formation *in vivo* evidently plays a leading role

in the mechanism of development of cardiovascular diseases [7]. Inhibition of TXA_2 biosynthesis in the platelets by trapidil and simultaneous activation of PGI_2 formation in the coronary arteries and also, possibly, in the heart tissue, are thus two mutually complementary processes whereby trapidil exerts its antiaggregating, coronary-dilating, and positive inotropic and chronotropic effects on the myocardium.

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TIME COURSE OF BLOOD ETHANOL IN RATS DURING ALCOHOL DEPENDENCE AND WITHDRAWAL

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During chronic alcohol intake both in animals and man, a considerable increase is observed in the activity of ethanol-oxidizing enzyme systems [5, 7]. The rapid disappearance of ethanol from the blood eliminates many of its toxic effects, and thus causes the development of metabolic tolerance, which in turn is the precursor of physical dependence on alcohol. Alcohol deprivation in the stage of dependence leads to a state of alcohol abstinence. According to one view [1], one of the mechanisms of formation of the abstinence syndrome is increased activity of ethanol-oxidizing enzyme systems in the period of alcohol deprivation.

The investigation described below accordingly was undertaken with the aim of studying the kinetics of ethanol as a parameter of the rate of its elimination in the stage of alcohol dependence and also during its withdrawal.

EXPERIMENTAL METHOD

Experiments were carried out on 25 noninbred male albino rats weighing 500-600 g, separated into four groups with six rats in each group. Animals of the first three groups were kept in individual cages with free choice between 15% ethanol solution and water for 8 months, the time required to form physical dependence on alcohol [2]. Animals of group 4, of the same age but with no contact with alcohol, served as the control. The time course of the blood alcohol of the animals of group 1 was studied 15 h after withdrawal, and in rats of groups 2 and 3, 2 and 7 days, respectively, after withdrawal.

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