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Coronary Responses to Dilating Substances and Competitive Inhibition by Theophylline in the Isolated Perfused Guinea Pig Heart *

Rolf Bünger, Francis J. Haddy**, and Eckehart Gerlach

Physiologisches Institut der Universität München

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Summary. Coronary dilation induced by infusion of adenosine, adenine nucleotides, dipyridamole, and papaverine was quantitated in the spontaneously beating isolated perfused guinea pig heart. Theophylline antagonized the effects of all the substances tested. The inhibition proved to be reversible and of a competitive type.

Single injections of ADP and ATP induced flow increases which were more rapid in onset and of greater magnitude than those due to equimolar amounts of adenosine. Lowering the perfusate temperature prolonged coronary responses to ADP and ATP more than those to adenosine.

Papaverine produced greater maximal dilation than adenosine. Theophylline inhibited papaverine-induced dilation less effectively than dilating responses to adenosine and other compounds. In the potassium arrested heart, the dilation caused by compound D 600 and papaverine was sensitive to the perfusate calcium concentration but that due to adenosine was unaffected. Dipyridamole, which was equipotent to adenosine in the non-arrested heart, became less potent than adenosine in the arrested heart.

The results favour the view that all of the substances tested induce coronary dilation per se and that their effects are not mediated by adenosine. The dilator response to papaverine is assumed to be the result of two effects, one of which is inhibited by theophylline, the other by high extracellular calcium.

Key words: Coronary Dilation — Adenosine — Adenine Nucleotides — Papaverine — Compound D 600 — Extracellular Calcium — Vascular Smooth Muscle.

Adenosine has been postulated to be involved in the coronary dilation caused by 5′-adenine nucleotides [2, 12] and to mediate the responses to coronary dilators, such as dipyridamole, hexobendine, and lidoflazine [16]. This concept is in agreement with the observations that injected ATP and 5′-AMP are partly dephosphorylated to adenosine during their passage through the heart [2, 11, 12] and that dilation due to dipyridamole, hexobendine, and lidoflazine can be inhibited by

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** Present address: Michigan State University, Department of Physiology, East Lansing, Michigan 48824, U.S.A.

theophylline [1,16,18], a compound which is known to also antagonize the vascular effects of adenosine [1,3,6,15,16,18].

Since the mechanism of the inhibition by theophylline has not been fully elucidated, it is not possible to decide whether adenosine actually functions as a mediator of the dilation produced by these substances or whether these compounds may act per se via a mechanism which can be blocked by theophylline.

In this study an attempt was made to distinguish between these possibilities. Coronary dilator responses to adenosine, adenine nucleotides, papaverine, and dipyridamole were therefore quantitated in the isolated guinea pig heart in the absence and presence of theophylline. The procedure allowed the determination of the type of antagonism between theophylline and the vasoactive substances tested. To further evaluate the mechanism of action of coronary dilating agents, the influence of temperature and extracellular calcium concentration on the vasodilator responses was examined.

Materials and Methods

Adenosine and adenine nucleotides (except 2′-AMP) were obtained from Boehringer Mannheim GmbH, Mannheim. Possible contaminants in the commercial preparations of the cyclic adenine nucleotides (2′,3′-AMP, 3′,5′-AMP) were removed by paper chromatography according to methods described elsewhere [10]. Dipyridamole was a gift from Dr. K. Thomae GmbH, Biberach a. d. Riß. Compound D 600 (α-isopropyl-α[(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl]-3,4,5-trimethoxy-phenylacetonitrile) was generously supplied by Knoll AG, Ludwigshafen. All other chemicals from E. Merck AG, Darmstadt, were of the highest available purity.

Stock solutions (1 mg/ml; theophylline: 20 mg/ml) of the vasoactive substances were prepared immediately before use. The compounds were dissolved either in 0.9% NaCl or, in the cases of 3′-AMP, 3′,5′-AMP, papaverine, dipyridamole, and theophylline, in 0.9% NaCl: ethanol = 1:1, v/v.

Spontaneously beating isolated guinea pig hearts were perfused according to the Langendorff technique using a non-recirculating system (hydrostatic pressure 75 cm H_2O). Perfusion medium was a modified Krebs-Ringer-bicarbonate solution containing 5.0 meq/l Ca^{2+}, fortified by pyruvate (2.0 mM) and glucose (5.0 mM). The perfusate was equilibrated with 5% CO_2 and 95% O_2 at 38° C, pH 7.41—7.45. The hemodynamic and metabolic stability as well as the reactivity of the coronary system in this preparation have been described in detail [5].

Infusion Studies. To elicit decreases in coronary resistance aliquots of the concentrated stock solutions were added to the perfusion fluid. When the increase in flow reached a steady state, coronary resistance was calculated from the ratio coronary perfusion pressure: mean coronary inflow. Perfusion pressure was taken from a calibration curve relating coronary orifice pressure to different flow rates through the perfusion system. Decreases in coronary resistance were expressed in percent of a response to 250 µg adenosine injected into the aortic cannula.

In hearts arrested with KCl (30 meq/l) pressure-flow relations were determined by stepwise increases of perfusion pressure from 40 to 82.5 mm Hg at high (7.5 meq/l) and low (1.5 meq/l) perfusate calcium concentrations. Perfusate osmolality was kept constant by adjusting the amount of NaCl.

Injection Studies. Small volumes (0.1 ml) of oxygenated perfusate containing 3.8 nmol of adenosine, ADP, and ATP, respectively, were injected into the aortic cannula 50 mm above the coronary orifice. Injection time (0.5 sec) was kept constant by use of an electromechanical injection apparatus. The following parameters of coronary inflow responses were measured: peak change of diastolic flow = maximal increase in diastolic inflow (ml/min · g wet weight); time to half recovery = time interval between onset of response and return to 50% of maximal diastolic flow change; latent period = time interval between injection artifact and the increase in either systolic or diastolic inflow. Left ventricular isovolumetric pressure changes and spontaneous heart rate were continuously recorded (Beckman Dynograph).

All data were statistically analyzed using the Student-*t*-test for unpaired, samples [14].

Results

A. Coronary Resistance Responses

Decreases in coronary resistance in unloaded hearts induced by infusion of various vasoactive substances are shown as dose response curves in Fig. 1. Adenosine, ADP, and ATP were the most potent compounds with almost identical activities. 5′-AMP was slightly less effective. The effects of the other adenine nucleotides decreased in the order 2′-AMP ⩾ 3′-AMP > 2′,3′-AMP > 3′,5′-AMP. Dibutyryl-cAMP (N^6-2′-

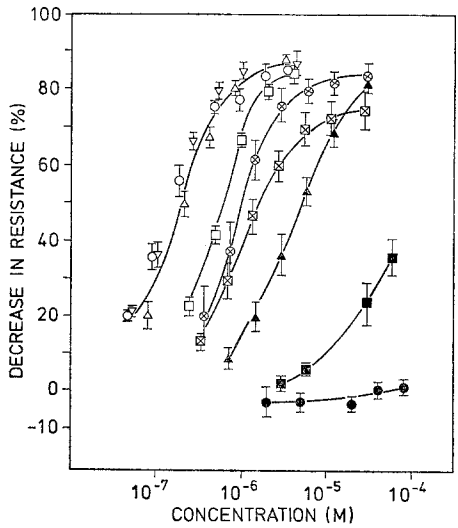

Fig. 1. Dose response relations for decreases in coronary resistance due to adenosine and adenine nucleotides in the isolated guinea pig heart. Mean values ± SEM from 4—8 experiments. Adenosine o——o, 5′-AMP □——□, ADP △——△, ATP ▽——▽, 2′-AMP ⊗——⊗, 3′-AMP ⊠——⊠, 2′,3′-AMP ▲——▲, 3′,5′-AMP ■——■, dibutyryl-cAMP ●——●

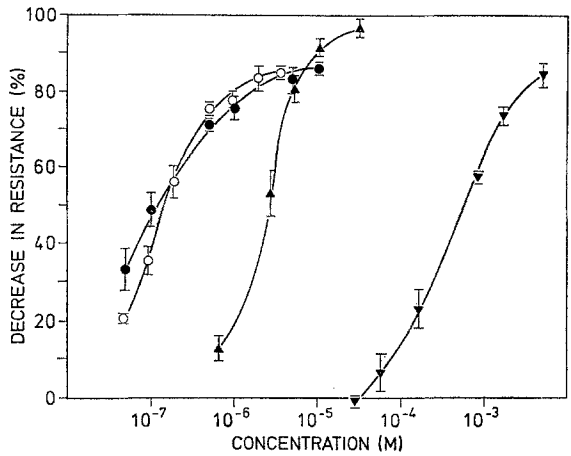

Fig. 2. Dose response relations for decreases in coronary resistance due to adenosine o——o, dipyridamole •——•, papaverine ▲——▲, and theophylline ▼——▼ in the isolated guinea pig heart. Mean values ± SEM from 6—8 experiments

O-dibutyryl-3′,5′-AMP) in concentrations less than 10^{-4} M was found to be vasoinactive.

Fig. 2 presents dose response curves for adenosine, dipyridamole, papaverine, and theophylline. The activities of adenosine and dipyridamole proved to be almost identical. Papaverine had to be used in higher concentrations than adenosine to cause comparable decreases in coronary resistance, but large doses of papaverine induced a greater reduction of resistance than high concentrations of adenosine. Vasodilating effects of theophylline could only be elicited by concentrations above 8×10^{-5} M.

In isovolumetrically beating hearts increases of coronary flow were associated with an acceleration of spontaneous heart rate ($\leqslant 10\%$) and an increase in left ventricular pressure ($\leqslant 30\%$). These alterations were most pronounced with theophylline in high concentrations ($> 10^{-3}$ M). Dibutyryl-cAMP did not significantly affect heart rate and left ventricular pressure.

B. Competitive Inhibition by Theophylline

When experimentally induced increases of coronary flow had reached a steady state, vasoinactive doses of theophylline ranging between 9×10^{-7} M and 5.5×10^{-5} M, were added to the perfusate. The resulting effects on coronary resistance are shown in Fig. 3, upper part. Increasing concentrations of theophylline progressively inhibited the effects of adenosine, ATP, dipyridamole, and papaverine, respectively. Conversely,

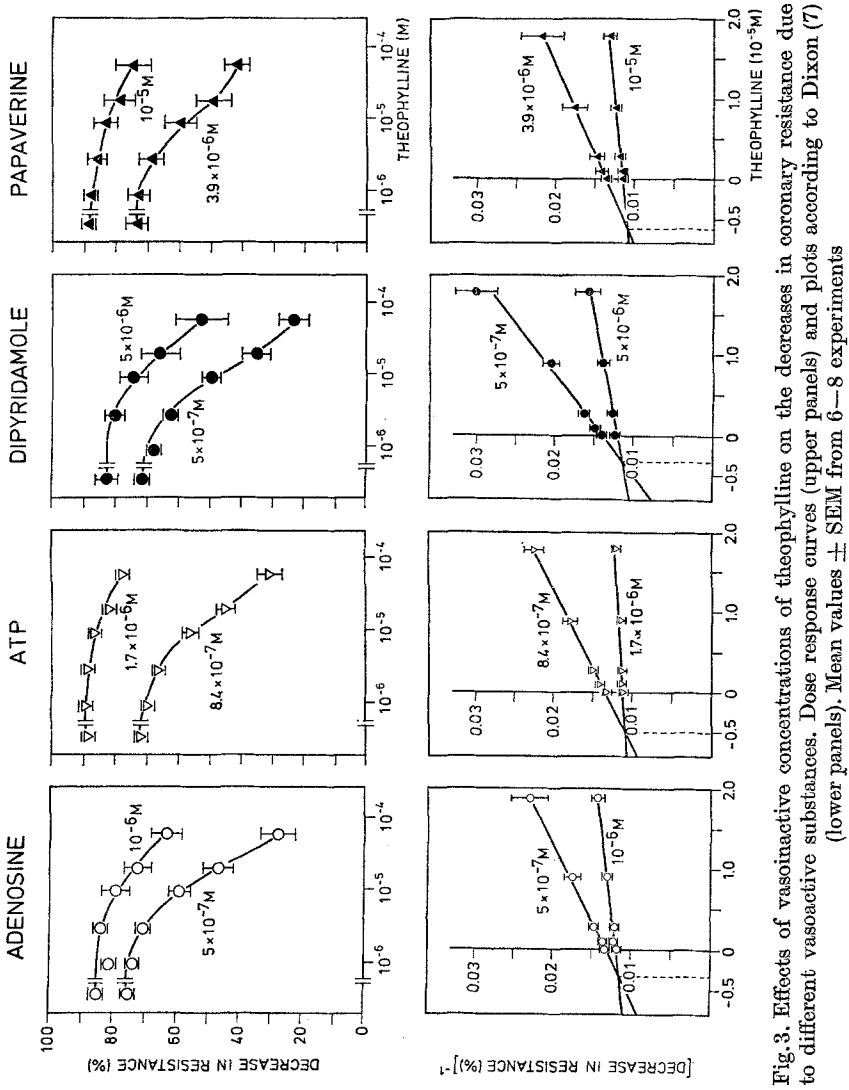

Fig. 3. Effects of vasoinactive concentrations of theophylline on the decreases in coronary resistance due to different vasoactive substances. Dose response curves (upper panels) and plots according to Dixon (7) (lower panels). Mean values ± SEM from 6—8 experiments

raising the concentrations of the vasodilators tended to overcome the antagonistic action of theophylline. Furthermore, the effects of theophylline proved to be reversible. The results therefore suggest an antagonism of a competitive type.

This assumption was confirmed by an analysis of the data according to Dixon [7], (Fig. 3, lower part). It is evident that the transformed

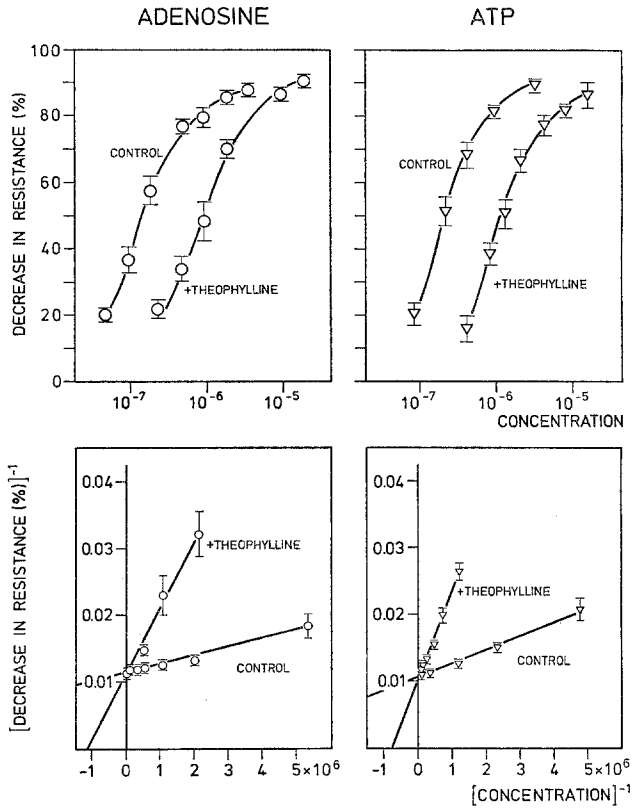

Fig. 4. Dose response relations for decreases in coronary resistance due to adenosine and ATP in the absence and presence of theopylline (5.5×10^{-5} M). Dose response curves (upper panels) and plots according to Lineweaver-Burk (lower panels). Concentration M/l. Mean values ± SEM from 6—8 experiments

data define pairs of straight lines which intersect left of the ordinate above the abscissa. Further evidence for competitive inhibition was obtained in additional experiments, in which dose response relations for adenosine and ATP were measured in the absence and presence of a constant concentration of theophylline (Fig. 4). By evaluating these data according to Lineweaver-Burk (Fig. 4, lower part) pairs of straight lines were obtained, which intersect on the ordinate.

Coronary dilation caused by papaverine proved to be less sensitive to the antagonistic action of theophylline than responses to other compounds. According to the data in Table 1 the resistance response to papaverine was inhibited only by 42%, whereas responses to equieffective

Table 1. Comparison of the inhibition by theophylline (5.5×10^{-5} M) of equieffective concentrations of coronary dilating substances in the isolated guinea pig heart. Mean values ± SEM from 4—6 experiments

	Decrease in resistance[a] %	Inhibition by theophylline[b] %
Papaverine (3.9×10^{-6} M)	72.5 ± 3.6	41.9 ± 3.4
Adenosine (5.0×10^{-7} M)	74.6 ± 1.7	63.9* ± 7.1
ATP (8.4×10^{-7} M)	72.2 ± 0.9	56.8* ± 4.4
3′-AMP (5.5×10^{-6} M)	77.0 ± 2.4	57.5** ± 2.8
Dipyridamole (5.0×10^{-7} M)	71.3 ± 1.6	67.6*** ± 4.9

[a] Calculated in percent of the resistance response to 250 µg adenosine.
[b] Calculated in percent of the resistance response in the absence of theophylline.
* $P < 0.025$, ** $P < 0.005$, *** $P < 0.0025$.

amounts of adenosine, ATP, 3′-AMP, and dipyridamole were reduced by more than 57%.

It is interesting to note that propranolol and atropin, which inhibited coronary dilation induced by isoprenaline and acetylcholine, respectively, did not influence the effects of adenosine, ATP, or dipyridamole. On the other hand, theophylline did not antagonize dilator responses to isoprenaline and acetylcholine.

C. Influence of Temperature

Table 2 summarizes the results from experiments in which three parameters of phasic coronary inflow were measured during responses to adenosine and 5′-adenine nucleotides. At 38° C the latent period of the adenosine response was longer than that of reactions to ADP and ATP. Furthermore, the peak increase in flow was smallest in the case of adenosine. Reduction of perfusate temperature increased the latent periods and reduced the peak flow responses, but the differences between the effects of the agents remained almost unchanged. Moreover, time to half recovery, which was similar for the three compounds at 38° C, increased more in the cases of ADP and ATP than in the case of adenosine at 26° C. Thus, the response to adenosine was slower in onset and less pronounced, and low perfusate temperature had a smaller effect on the time to half recovery.

D. Influence of Calcium

Pressure-flow relations in the presence of various dilator substances were determined in hearts which had been equilibrated for 30 min with

Table 2. Comparison of the effects of equimolar (3.8 nmol in 0.1 ml) amounts of adenosine, ADP, and ATP on the coronary flow parameters at normal and reduced perfusate temperature in the isolated guinea pig heart. Mean values ± SEM from 5 experiments

	Latent period sec		Peak change of diastolic flow ml/min · g		Time to half recovery sec	
	38 °	26 °	38 °	26 °	38 °	26 °
Adenosine	2.4 ± 0.2	3.9 ± 0.2	7.3 ± 0.5	4.7 ± 0.3	14.5 ± 1.2	29.5 ± 2.5
ADP	1.5** ± 0.1	2.6*** ± 0.1	9.6*** ± 0.7	6.5*** ± 0.4	16.8** ± 0.7	46.3*** ± 3.0
ATP	1.9* ± 0.2	3.2* ± 0.1	9.0*** ± 0.6	6.1*** ± 0.3	15.3 ± 3.0	40.3*** ± 3.0

* $P < 0.025$, ** $P < 0.0025$, *** $P < 0.0005$ relative to adenosine.

Table 3. Pressure-flow relation at high and low perfusate calcium concentrations in the potassium arrested isolated guinea pig heart. Mean values ± SEM from 26 experiments

Perfusion pressure mm Hg	Coronary flow (ml/min · g)*		Significance
	Ca^{2+} 7.5 meq/l	Ca^{2+} 1.5 meq/l	
40	0.72 ± 0.05	0.92 ± 0.10	$P < 0.05$
55	1.05 ± 0.08	1.49 ± 0.16	$P < 0.01$
82.5	1.77 ± 0.13	2.39 ± 0.29	$P < 0.025$

* Dry weight in percent of wet weight was 8.9 ± 0.15 % (Ca^{2+} 7.5 meq/l) and 9.0 ± 0.18 % (Ca^{2+} 1.5 meq/l).

high (7.5 meq/l) and low (1.5 meq/l) calcium concentrations. The effects of calcium-induced changes of cardiac contractility and subsequent alterations of extravascular pressure on coronary resistance were abolished by arresting the hearts with potassium.

Table 3 presents pressure-flow relations at the two levels of calcium in the absence of the vasodilating substances. The addition of compound D 600, papaverine, and adenosine produced vasodilation, but dipyridamole even in high concentrations proved to be almost vasoinactive (Fig. 5), though it was equipotent to adenosine in the non-arrested heart (see Fig. 2 and Table 1). The extracellular calcium concentration had a remarkable effect on the dilation caused by D 600 and papaverine but did not influence that produced by adenosine.

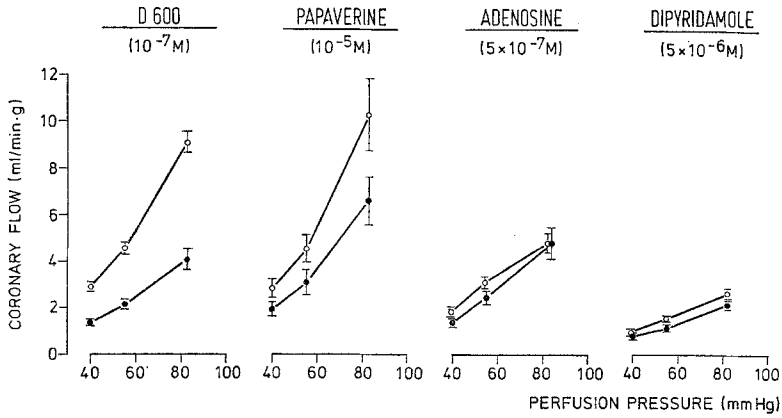

Fig. 5. Effects of high (•——•, 7.5 meq/l) and low (o——o, 1.5 meq/l) perfusate calcium concentrations on coronary pressure-flow relation in the presence of different vasoactive substances. Heart arrested with potassium. Mean values ± SEM from 4—6 experiments

Discussion

The metabolic and hemodynamic stability and the coronary reactivity of the preparation used (see reference [5]) made it possible to perform studies which contribute to further clarify the mechanism by which various vasoactive substances induce coronary dilation.

The most interesting finding seems to be the observation that the inhibition by theophylline of the coronary effects of adenosine and dipyridamole [1, 3, 6, 15, 16, 18] is of a competitive type and that a competitive antagonism also exists between theophylline and the coronary responses to ATP, 3′-AMP, and papaverine. These results suggest that adenosine induces coronary dilation by a mechanism which seems to be not different from that responsible for the effects of ATP, 3′-AMP, dipyridamole or papaverine. Such a mechanism can be also held responsible for the dilator responses to other adenine nucleotides (2′-AMP, 2′,3′-AMP, 3′,5′-AMP), since their effects are likewise inhibited by theophylline (unpublished observations). As indicated by the positions of the dose response curves in Figs. 1 and 2 the sensitivity of this mechanism is similar and greatest for adenosine, 5′-adenine nucleotides, and dipyridamole, whereas it is less in the cases of the other adenine mononucleotides seemingly dependent on the structure of these molecules. In addition, since adenine nucleotides cannot penetrate across normal cell membranes, these substances may be assumed to act on or near the surface of the membrane of the coronary smooth muscle cell.

On the other hand, participation of adenosine in the coronary responses to the adenine nucleotides [2, 12] and dipyridamole (see for instance [16]) could also explain the competition between these compounds and theophylline. However, dilator responses to injected ADP and ATP appear sooner and are of greater magnitude than those to equimolar doses of adenosine. Since these differences remain the same and the duration of the responses to ADP and ATP is augmented to a greater extent at lower perfusate temperature, it seems unlikely that dephosphorylation of the nucleotides to adenosine during the passage through the heart [2, 11, 12] is a necessary prerequisite for the dilator activity of these substances. Consequently, the nucleotides can be assumed to cause vasodilation per se. Also in the renal vascular system ATP exhibits hemodynamic effects which cannot be mediated by adenosine, since it causes vasodilation whereas adenosine induces vasoconstriction [17]. Furthermore, latent periods subsequent to injections of adenosine and equieffective doses of papaverine proved to be identical both at normal and reduced temperature (unpublished data). These observations, too, do not favour the view that adenosine participates in the coronary dilator response to papaverine. Thus, the competitive type of inhibition by theophylline can be explained by the concept of a coronary dilator mechanism which is common to adenosine, adenine nucleotides, dipyridamole, and papaverine and which is blocked by theophylline.

The perfusate calcium concentration influences the dilator response to papaverine but not that to adenosine. This apparent disagreement with the fact that theophylline acts as an antagonist of the effects of both substances might be explained by the assumption that dilation induced by papaverine is the result of dual effects of this substance, one of which is influenced by theophylline, the other by extracellular calcium. Such a concept is compatible with the findings that papaverine induces a greater maximal dilator response than adenosine and that theophylline inhibits less effectively vasodilation due to papaverine than to adenosine and other compounds. This, too, suggests that the total dilatory response to papaverine is the result of a calcium sensitive and a theophylline sensitive process.

Dilating agents like iproveratril and its derivative D 600 have been shown [8, 13] to inhibit calcium penetration across cellular membranes. It is the calcium antagonistic effect of these compounds which is assumed [9] to be directly responsible for the reaction of the coronary vessels. In support of this concept is our finding that the coronary dilation induced by compound D 600 is influenced by the extracellular calcium concentration. Since calcium has a similar but smaller influence on papaverine-induced dilation it is possible that the response to papaverine

results in part via the same mechanism as that responsible for the effect of compound D 600. On the other hand, there is no congruency between the mechanism of action of compound D 600 and adenosine since the extracellular calcium concentration does not influence the dilator response to adenosine. Despite these differences it must be assumed that adenosine and the other compounds finally induce vasodilation by decreasing the effective calcium concentration in the environment of the troponin-actomyosin-ATPase system, as has been recently outlined by Bohr [4]. The means, however, by which such a diminution of intracellular calcium is actually brought about needs further clarification.

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References

1. Afonso, S.: Inhibition of coronary vasodilating action of dipyridamole and adenosine by aminophylline in the dog. Circulat. Res. **26**, 743 (1970)
2. Baer, H. P., Drummond, G. J.: Catabolism of adenine nucleotides by the isolated perfused rat heart. Proc. Soc. exp. Biol. (N.Y.) **127**, 33 (1968)
3. Bittar, N., Pauly, T. J.: Myocardial reactive hyperemia responses in the dog after aminophylline and lidoflazine. Amer. J. Physiol. **220**, 812 (1971)
4. Bohr, D. F.: Vascular smooth muscle updated. Circulat. Res. **32**, 665 (1973)
5. Bünger, R., Haddy, F. J., Querengässer, A., Gerlach, E.: An isolated guinea pig heart preparation with in vivo like features. Pflügers Arch. **353**, 317 (1975)
6. Dietmann, K., Roesch, E., Schaumann, W., Juhran, W.: The effect of cyclic adenosine- and guanosine 3′,5′-monophosphate on the normal and drug-increased coronary blood flow. Biochem. Pharmacol. **21**, 2193 (1972)
7. Dixon, M.: The determination of enzyme inhibitor constants. Biochem. J. **55**, 170 (1953)
8. Fleckenstein, A.: Pathophysiologische Kausalfaktoren bei Myokardnekrose und Infarkt. Wien. Z. inn. Med. **52**, 133 (1971)
9. Fleckenstein, A., Grün, G.: Prinzipielles zur Wirkung von Ca^{++}-Antagonisten auf die bioelektrische und mechanische Funktion glatter Muskelzellen. In: Vascular smooth muscle, p. 62. Berlin-Heidelberg-New York: Springer 1972
10. Gerlach, E., Deuticke, B., Dreisbach, R. H., Rosarius, C. W.: Zum Verhalten von Nucleotiden und ihren dephosphorylierten Abbauprodukten in der Niere bei Ischämie und kurzzeitiger postischämischer Wiederdurchblutung, Pflügers Arch. ges. Physiol. **278**, 296 (1963)
11. Hopkins, S. V.: The action of ATP in the guinea pig heart. Biochem. Pharmacol. **22**, 335 (1973)
12. Jacob, M. I., Berne, R. M.: Metabolism of purine derivatives by the isolated cat heart. Amer. J. Physiol. **198**, 322 (1960)
13. Kohlhardt, M., Bauer, B., Krause, H., Fleckenstein, A.: Differentiation of transmembrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. Pflügers Arch. **335**, 309 (1972)
14. Richterich, R.: Klinische Chemie, Theorie und Praxis. Basel: S. Karger 1971
15. Schaumann, W., Juhran, W., Dietmann, K.: Antagonismus der Kreislaufwirkungen von Adenosin durch Theophyllin. Arzneimittel-Forsch. **20**, 372 (1970)

16. Scholtholt, J., Nitz, R. E., Schraven, E.: On the mechanism of the antagonisti action of xanthine derivatives against adenosine and coronary vasodilators. Arzneimittel-Forsch. **22**, 1255 (1972)
17. Scott, J. B., Daugherty, R. M., Jr., Dabney, J. M., Haddy, F. J.: Role of chemical factors in regulation of flow through kidney, hind-limb, and heart. Amer. J. Physiol. **208**, 813 (1965)
18. Spieckermann, P. G., Hellberg, K., Kettler, D., Reploh, H. D., Strauer, B.: Hemmung der coronardilatierenden Wirkung von Adenosin, Dipyridamol, Hexobendin und ASTA C 4898 durch Aminophyllin (Theophyllin-Äthylendiamin). Pflügers Arch. **312**, R 15 (1969)

Authors' address:
Physiologisches Institut der Universität
D-8000 München 2, Pettenkoferstr. 12
Federal Republic of Germany