Biochemical Pharmacology, Vol. 35, No. 24, pp. 4401–4407, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

CALCIUM ANTAGONISTS STIMULATE PROSTAGLANDIN SYNTHESIS BY CULTURED RAT CARDIAC MYOCYTES AND PREVENT THE EFFECTS OF HYPOXIA

BRIGITTE ESCOUBET,* GENEVIÈVE GRIFFATON, JANE-LISE SAMUEL and PAUL LECHAT
Institut de Pharmacologie, 15 Rue de l'école de Médecine, and INSERM U 127, Hôpital Lariboisière, Paris, France

(*Received* 30 *January* 1986; *accepted* 25 *June* 1986)

Abstract—The effect of three calcium antagonists on the synthesis of prostacyclin (PGI2, assayed as 6-Keto-PGF1alpha) and PGE2 by cultured rat cardiac myocytes and fibroblasts was investigated. In myocytes only, bepridil, diltiazem and verapamil (10^{-9} to 10^{-7} M) stimulated PGs synthesis by two- to three-fold, dose-dependently. At a concentration of 10^{-6} or 10^{-5} M the intensity of the stimulation of PGI2 and PGE2 decreased. Cobalt chloride (2×10^{-3} M) did not change PGs synthesis (pg/mg of protein/30 min; means ± SE, N = 10; PGE2: 365 ± 59 and 463 ± 89 treated vs controls; PGI2: 824 ± 214 and 799 ± 143 treated vs controls). After 30 min exposure of myocytes to hypoxic conditions (glucose-free medium and low PO2), the glycogen content was half that of the controls ($P < 0.001$), ATP content did not change and PGI2 and PGE2 synthesis increased (×1.5, $P < 0.05$). When applied to myocytes 30 min before inducing hypoxia, the three calcium antagonists stimulated PGs synthesis by three- to seven-fold at maximal effect, and bepridil (10^{-8} M) or diltiazem (10^{-7} M) prevented the hypoxia-induced decrease in glycogen content. With 10^{-5} M drug concentration, the effect on PGs was not significant, except for the effect of bepridil on PGI2 ($P < 0.05$). It is concluded that therapeutic concentrations of calcium antagonists simultaneously prevent the decrease in myocyte glycogen induced by hypoxia and stimulate PGs synthesis by myocytes.

Among prostaglandins (PGs), prostacyclin (PGI2) is a potent vasodilating agent [1] which may play a role in the physiological regulation of myocardial blood flow [2] and protect myocardium against ischemia [3]. Calcium antagonists, which are potent vasodilating agents [4], are used for the treatment of angina pectoris [5] and have been shown, in isolated heart and in animal models, to protect myocardium against ischemic injuries [6–8]. These effects are believed to be the consequence of calcium channel blockade and of the subsequent coronary vessel dilation. Whether or not calcium antagonists exert a direct protection of myocardium against ischemia remains controversial [9]. The similarity of the cardiac effects of PGs and calcium antagonists suggests that alterations of arachidonic acid metabolism could be instrumental in the effects of the latter. Indeed, verapamil (10^{-5} to 10^{-4} M) was shown not only to decrease thromboxane synthesis by platelets and to inhibit platelet aggregation [10] but also to increase the circulating PGs [11] and the production of PGs by isolated tissues [12].

We have investigated the effect of three calcium antagonists, bepridil, diltiazem and verapamil, on the synthesis of PGs by cardiac cells in culture, and on the effects of hypoxia on the cells.

* Address for reprints: Dr Brigitte Escoubet, Faculté de Médecine X. Bichat, INSERM U 251, 16 Rue H. Huchard, 75018 Paris, France.

MATERIALS AND METHODS

Cell culture. Heart myocytes and fibroblasts were cultured from 3-day-old Sprague–Dawley rats (Charles River, France). Ventricular cells were dissociated with porcine trypsin (Choay Lab., France) and myocytes were separated from fibroblasts by differential plating [13]. Cultures were grown for 3 days in Minimal Essential Medium (MEM) (Eurobio, France) containing 10% new-born calf serum (Eurobio, France).

Characterization of the culture, as myocyte or fibroblast, was made by indirect double immunofluorescence with specific antibodies against myosin and tubulin [14]. For immunofluorescence studies, cells were grown on coverglasses and exposed successively to both antibodies, fluorescein-labeled anti-rabbit immunoglobulins G and to rhodamin-labeled anti-guinea pig immunoglobulins G [15]. The same field of the culture dish was observed under a fluorescence microscope (Leitz Dialux) to assess the preparation purity: the cultures, when used, contained at least 90% of the corresponding cell type (Fig. 1).

Experimental procedure. All procedures were carried out at 30° on the fourth day of culture. Attached cells were washed three times with MEM and left resting for 1 hr. The medium was then replaced by 2 ml of fresh MEM and incubated for two or three periods of 30 min. After each incubation, 1 ml of the incubate was removed for assay of PGs and replaced by 1 ml of fresh MEM or

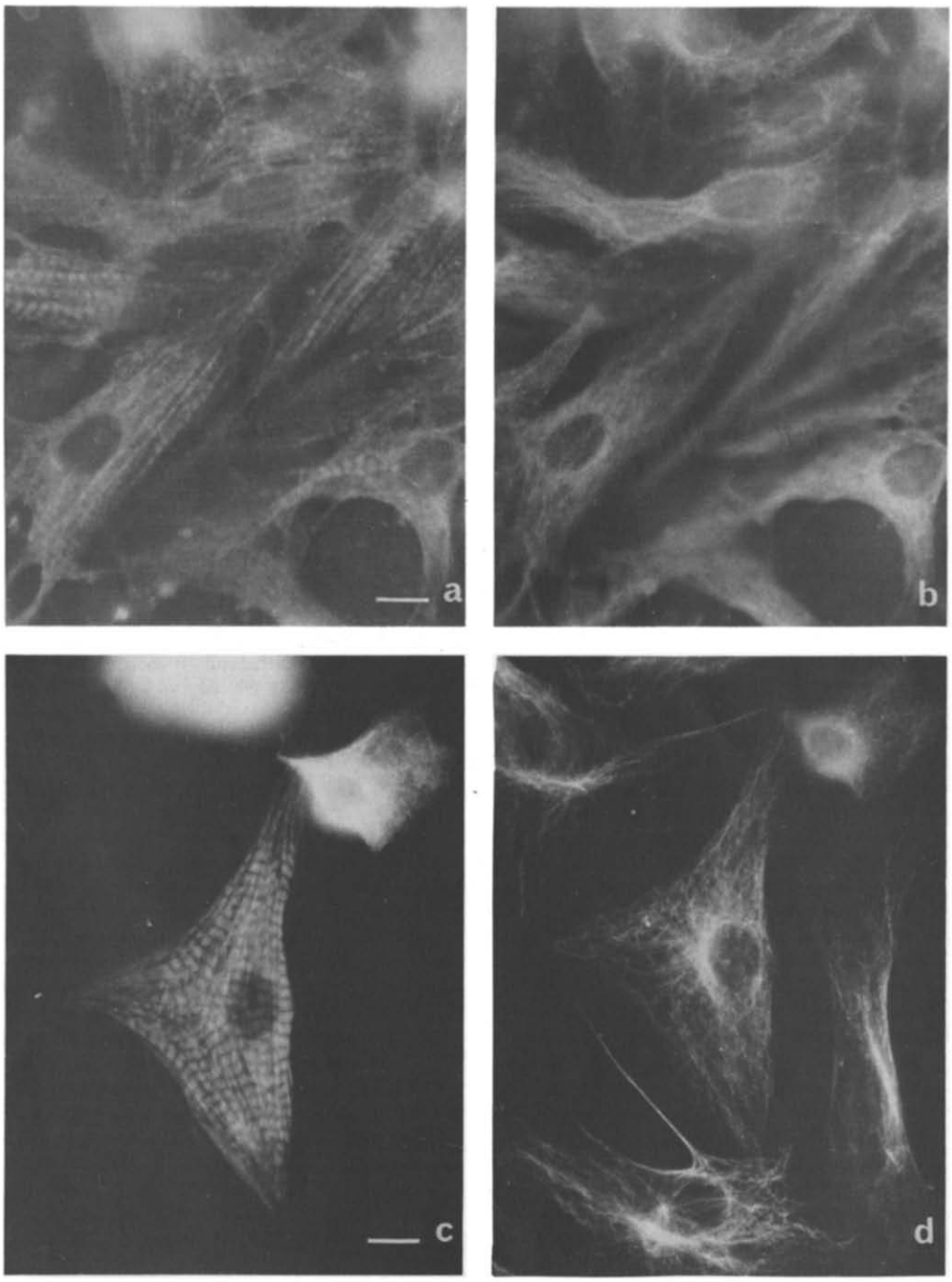

Fig. 1. Characterization of myocyte and fibroblast cultures by indirect double immunofluorescence with anti-myosin and anti-tubulin antibodies. The figure shows the same field of cells labeled with the two fluorescent markers in a myocyte culture (a and b) and in a fibroblast culture (c and d). Tubulin is present in the two types of cell (b, d) whereas myosin is specific for myocytes (a, c). Bar = 10 μm.

MEM containing the drug. The removed medium was rapidly frozen and stored at −20° until assay. The first incubation was performed under control conditions and the additional two periods under control or treated conditions. The amount of PGs actually produced during the considered 30 min of incubation was calculated by subtracting the amount of PGs synthetized during the previous incubation periods from the total amount assayed, as previously described [16] in renal glomerular cell cultures. The cells were washed with 0.9% NaCl, harvested with a rubber policeman and assayed for protein content [17].

Hypoxic conditions were achieved by incubating the cells in glucose-free MEM (Eurobio, France) saturated with 95% N_2, 5% CO_2 in order to decrease

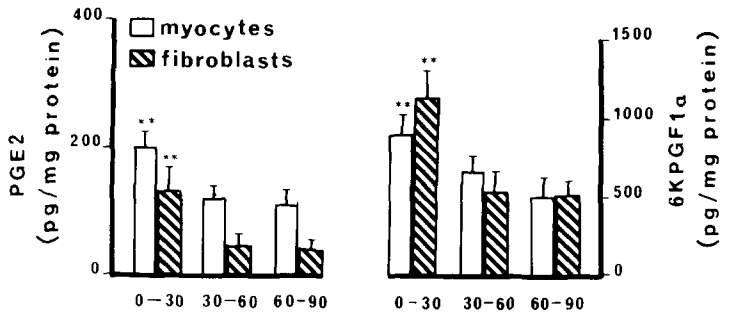

Fig. 2. Amounts of PGs synthetized by heart cells in culture during three successive 30-min periods of incubation. Data are means ± SE; *P < 0.05, ** P < 0.01; N = 25 for myocytes and N = 14 for fibroblasts.

the PO_2 (24 ± 1.4 mmHg versus 121 ± 2.6 mmHg in controls, P < 0.001). Gassing was performed through a rubber cap to allow sampling without opening the vial. Controls were gassed with 20% O_2, 75% N_2, 5% CO_2.

Verapamil (Knoll Pharmaceutical Co.), diltiazem hydrochloride (LERS Synthelabo, France) and bepridil hydrochloride (Riom CERM Labs., France) were dissolved in MEM, kept protected from the light and were added to the cells 30 min before inducing hypoxia. Cobalt chloride (Sigma Chemical Co.), dissolved in MEM, was used at a concentration of 2×10^{-3} M. Indomethacin (Sigma Chemical Co.) was dissolved in ethanol and further diluted in MEM, BW 755c (Wellcome Labs.) was dissolved in MEM.

In separate experiments, 0.5 μCi of ^{14}C-arachidonic acid (58 mCi/mmol, the Radiochemical Centre Amersham, U,K.) was added to the cells in a 3 μl volume of ethanol. The cells were incubated for a single period of 30 min.

Prostaglandin assay. In the experiments in which ^{14}C-arachidonic acid was used, PGs were extracted from the acidified incubation medium, the extract purified by silicic acid chromatography [18] and the fraction containing PGs used for reverse-phase HPLC. PGs were separated with a C18 micro Bondapack column (Waters Assoc., 250 mm, 10 μ particle size) with a mobile phase composed of acetonitrile/benzene/acetic acid/water (49.6/0.4/0.2/49.6, v/v) using a 2.0 ml/min flow rate (Gilson 302 pump). Fractions were collected every minute and counted for radioactivity by liquid scintillation (Packard M 4430, set up for dual label count). PGs were identified by the migration of the corresponding tritiated standard (150 Ci/mmol, the Radiochemical Centre, Amersham, U.K.) added before extraction.

In all other experiments, PGs were assayed by specific radioimmunoassay [18] in unextracted incubation medium. PGE2 and 6KetoPGF1alpha, the stable metabolite of PGI2, were assayed with antibodies from Institut Pasteur (Paris, France). Anti-6KPGF1alpha antibody cross-reacted with PGE2 (4%), PGE1 (13%) and dihydroPGF1alpha (4%), antiPGE2 antibody cross-reacted with PGE1 (10%) and 15KPGE2 (13%). The cross-reactivity of both antibody with other PGs was below 1%. MEM, bepridil, diltiazem, verapamil and cobalt chloride did not cross-react with the radioimmunoassay. Results were expressed as pg/mg of protein/30 min.

Glycogen and ATP assay. After incubation, cells were scraped off on ice and assayed for glycogen [19] or ATP cell content [20].

Statistical analysis. The two-tailed paired *t*-test was used to compare the amount of PGs produced during treated and untreated periods. One-way variance analysis was used in other comparisons.

RESULTS

Synthesis of PGs by cardiac cells in culture

The chromatography disclosed that myocytes and fibroblasts produced PGI2, as assayed by its stable metabolite 6-Keto-PGF1alpha, and PGE2 from ^{14}C-arachidonic acid. As quantified by radioimmunoassay, myocytes produced significantly greater amounts of PGE2 than fibroblasts (Fig. 2, P < 0.05), and similar amounts of PGI2. The synthesis of PGI2 and PGE2 by myocytes and fibroblasts was decreased by 70% after 1 hr incubation with indomethacin (10^{-6} M), whereas BW755c (40 mg/l) did not change the synthesis of PGs.

In controls, the amount of PGI2 and PGE2 produced during 30 min decreased by 55 ± 5% when the cells were incubated for a second 30-min period. No further decrease in the synthesis of PGs was observed during a third incubation of 30 min (Fig. 2). The

Table 1. Effect of cobalt chloride (2 mM) on PGs synthesis by cardiac myocytes and fibroblasts in culture

		Control	$CoCl_2$
PGE2			
Myocytes,	N = 10	463 ± 89	365 ± 58
Fibroblasts,	N = 16	42 ± 3.2	40 ± 3.7
PGI2			
Myocytes,	N = 10	799 ± 143	824 ± 214
Fibroblasts,	N = 10	296 ± 38	214 ± 33

Data are expressed as pg/mg of protein/30 min (means ± SE). Controls are the control values expected for the corresponding incubation time.

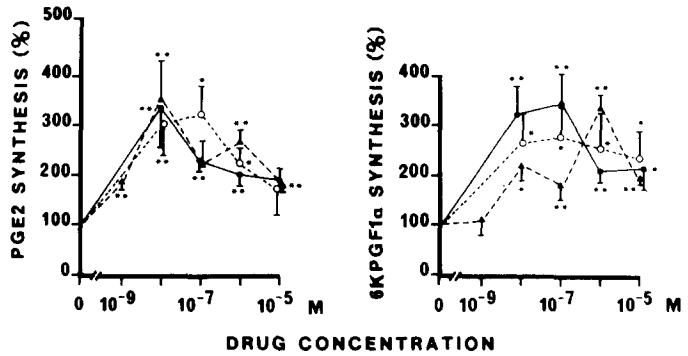

Fig. 3. Effect of bepridil, diltazem and verapamil on the synthesis of PGs by cultured rat cardiac myocytes under normal conditions. Results are shown as the percentage of the value expected for the corresponding incubation under control conditions. Data are means ± SE; N = 4 to 7: ▲, bepridil; ○, diltazem; ●, verapamil; **P < 0.01, as compared with the paired controls.

actual amount of PGs produced during the second or the third period of incubation was compared with the amount of PGs expected to be produced during the corresponding incubation period under control conditions.

Effects of calcium antagonists on the synthesis of PGs

At a concentration of 2×10^{-3} M cobalt chloride did not change the rate of synthesis of PGs either by myocytes or by fibroblasts (Table 1).

In contrast, the synthesis of PGs by myocytes was increased by bepridil, diltiazem and verapamil with a biphasic effect (Fig. 3). The maximal effect was a two- to three-fold increase and the stimulation of the synthesis of PGs decreased progressively when myocytes were incubated with higher concentrations of calcium antagonists. The calcium antagonists did not significantly affect the synthesis of PGs by fibroblasts.

Cobalt chloride (10^{-3} M) or bepridil, diltiazem and verapamil, at concentrations equal to or greater than 10^{-7} M, abolished the spontaneous beating activity of myocytes (213 ± 59 beats/min under control conditions), whereas at 10^{-8} M no significant change in the spontaneous beating activity was observed in myocytes (197 ± 28 beats/min).

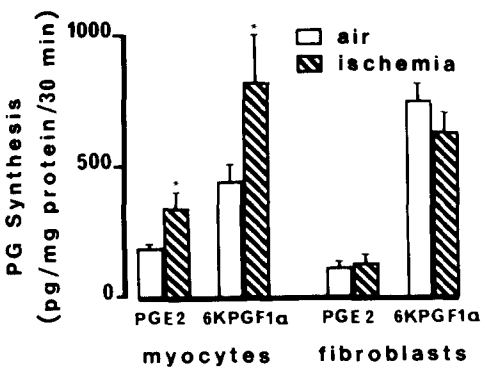

Fig. 4. Effect of hypoxia on synthesis of PGs by cardiac myocytes and fibroblasts. *P < 0.05, as compared with the paired value expected for the corresponding incubation under control conditions. Data are means ± SE; N = 33 for myocytes and N = 45 for fibroblasts.

Effects of hypoxia

In myocytes, glycogen content was significantly decreased by hypoxia whereas no significant change was found in fibroblasts (17.2 ± 3.8 mg/g of protein in controls vs 13.8 ± 0.17 mg/g of protein in hypoxic cells; N = 4) (means ± SE). ATP content did not change in myocytes or in fibroblasts (2.4 ± 0.05 nmol/mg of protein in control myocytes and 2.23 ± 0.17 nmol/mg of protein in hypoxic cells; N = 4). Under hypoxia, the spontaneous beating activity of myocytes was abolished.

In myocytes, hypoxia induced a significant increase in the production of PGs, whereas no change was observed in fibroblasts (Fig. 4).

Effects of calcium antagonists under hypoxia

The treatment of myocyte cultures with bepridil, diltiazem or verapamil before inducing hypoxia increased the synthesis of PGs, as compared with untreated cells under control conditions (Fig. 5). The effect of the calcium antagonists on PGs was qualitatively similar to that on cells gassed with air. Nevertheless, the effect on PGs of 10^{-6} M of bepridil was greater under hypoxia than under normal conditions (Table 2) and the stimulation of the synthesis of PGE2 was significantly greater during hypoxia than in control conditions with diltiazem or verapamil (10^{-8} M) (Table 2). With higher concentration of calcium antagonists (10^{-5} M), the synthesis of PGs was not significantly different from controls except for PGI2 with bepridil ($P < 0.05$).

The treatment of myocytes with bepridil (10^{-8} M) or diltiazem (10^{-7} M) prevented the glycogen depletion induced by hypoxia (Table 3).

Under hypoxia, the PGs synthesized by fibroblasts were not changed by calcium antagonists.

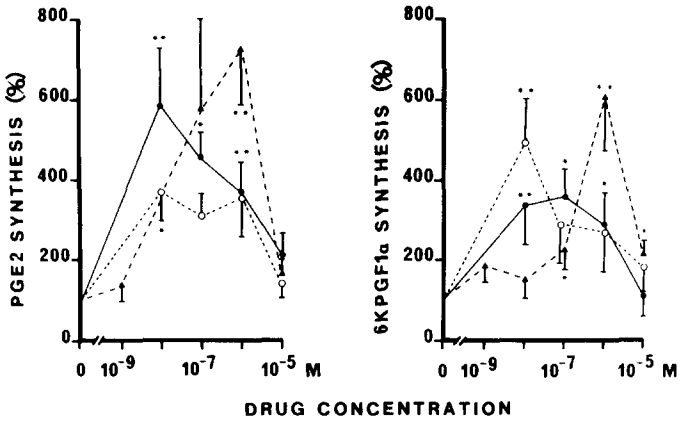

Fig. 5. Effect of bepridil, diltiazem and verapamil on synthesis of PGs by cardiac myocytes. Results are shown as the percentage of the value expected for the corresponding incubation under control conditions. Data are means ± SE; N = 4 to 7: ▲, bepridil; ○, diltiazem; ●, verapamil. *P < 0.05, **P < 0.01, as compared with the paired controls.

DISCUSSION

The results show that calcium antagonists stimulate the synthesis of PGI2 and PGE2 cardiac myocytes in culture under normal conditions and also under hypoxia. Treatment of myocytes with calcium antagonists prevented the depletion of glycogen induced by hypoxia.

The synthesis of PGs by cardiac tissues has been previously described in cultured rat cardiac cells with the detection of PGI2 and PGE2 [21, 22] and in human heart homogenates [23, 24]. These PGs are potent vasodilating and platelet antiaggregating agents [25]. Cardiac PGs originate from endothelial cells [26] but also from myocytes and fibroblasts [21, 22] and PGs are believed to modulate myocardial blood flow [2] and to protect the myocardium against ischemia [3, 27]. Since some of these studies were primarily qualitative, we quantified the production of PGs by heart cells *in vitro*.

We have studied the modification of the synthesis of PGs by calcium antagonists in the hypothesis that an alteration of the PGs synthesis might be instrumental in the cardiac actions of these drugs. Indeed, our results show that calcium antagonists stimulate the synthesis of PGs in myocytes. Moreover, the results show that the concentration of calcium antagonists which produces the maximal effect on the synthesis of PGs, *in vitro*, is close to the therapeutic plasma concentrations (10^{-8} to 10^{-7} M) [28, 29] suggesting a clinical relevance of the alteration of PGs by calcium antagonists.

The biphasic effect of the calcium antagonists, depending on the drug concentration, has been observed in other studies. In this context, it is worth mentioning recent studies [30, 31], in which it was shown that dihydropyridines (Bay K 8644 and nifedipine) have the opposite effect on cardiac inotropism depending on the drug concentration: negative inotropism with high concentration and positive inotropism or no change with low concentrations. Similar change in the cellular drug action might be involved in the alteration of cardiac PGs by low and high concentrations of the studied calcium antagonists. Moreover, the concentrations of calcium antagonists required to interfere with calcium transport *in vitro* are often much higher (10^{-6} M or more) [32, 33] than those increasing the synthesis of PGs and greater than those achieved during clinical use [34]. The discrepancy between the calcium antagonist concentration inhibiting calcium transport through the membrane and that promoting the synthesis of PGs, suggests that the latter effect might be independent of calcium entry blockade. Moreover, the lack of effect of cobalt chloride used at con-

Table 2. Comparison of the effect of calcium antagonists on myocyte PGs synthesis under control and hypoxic experimental conditions

		Air	Hypoxia
Bepridil (10^{-6} M)	PGE2	347 ± 47	706 ± 50*
	6KPGF1 α	1444 ± 257	2400 ± 407†
Diltiazem (10^{-8} M)	PGE2	462 ± 107	803 ± 167*
Verapamil (10^{-8} M)	PGE2	320 ± 130	493 ± 191*

Data are expressed as pg/mg of protein/30 min, as the mean ± SE, N = 4 to 7; *P < 0.05, †P < 0.01, as compared to the paired control.

Table 3. Effect of bepridil (10^{-8} M) and diltiazem (10^{-7} M) on myocyte glycogen content under control conditions and under hypoxia when the drugs were applied to the cells 30 min before hypoxia

		Air	Hypoxia
—	(N = 7)	7.2 ± 0.56	2.3 ± 0.75*
Bepridil	(N = 4)	7.5 ± 0.6	8.1 ± 0.88
Diltiazem	(N = 4)	8.3 ± 1.6	10.1 ± 1.73

Results are expressed as mg/g of protein, means ± SE; *$P < 0.001$.

centrations known to inhibit the calcium influx through the calcium channels [35] is an argument against the direct responsibility of the blockade of the calcium channel by calcium antagonists in the effects of these drugs on the synthesis of PGs. Further investigation is needed for establishing the interactions between calcium movement, calcium antagonists and the synthesis of PGs. Similarly, the decrease in the beating rate of the myocytes does not seem to be directly responsible for the observed effect on the synthesis of PGs by calcium antagonists as cobalt chloride does not alter the synthesis of PGs, whereas it stops cells beating.

The second part of our study dealt with the effect of calcium antagonists during hypoxia. The results of the present study show that our hypoxic conditions induced moderate alteration of the cultured cells as ATP content was maintained whereas glycogen decreased. Hoerter and Opie [36] have described the particular resistance to hypoxia of hearts isolated from new-born rabbits or rats, as compared with adults. They found that the high glycogen content of new-born heart cells was responsible for the preservation of the cellular ATP content during hypoxia by increasing the glycogenolysis [37]. The experimental conditions in our study might thus represent a model for *in vitro* study of moderate hypoxic myocardial injury and disclose the increased synthesis of PGs by myocytes under hypoxia as a possible adaptive mechanism of myocardium to hypoxia.

The protection of the myocardium against ischemia by calcium antagonists has been described in isolated hearts [7] or in *in vivo* models [8] and is believed to be the consequence of the vascular action of the drugs [4, 9]. The protection of the myocardium against ischemia by calcium antagonists was also found in our study. Despite the similarity between our observation and that obtained from *in vivo* models, the interpretation may be different: in our study the effect of calcium antagonists cannot be mediated through vascular effects of the calcium antagonists, as no vascular structure was present in the culture. The decrease in the cell beating frequency induced by calcium antagonists has been said to play a part in the protection of the myocardium against hypoxia by calcium antagonists [38]. This effect is unlikely to be the reason for the observed myocyte protection against hypoxia, because hypoxia itself decreases the myocyte beating rate to a similar extent. Another possible explanation for the protection of myocytes against hypoxia by calcium antagonists might be the stimulation of the synthesis of PGs. PGs, and particularly PGI2, are known to have such an effect, either in isolated heart [3, 39] or in clinical situations [40]. The possible role of PGs in the protection of the myocardium by calcium antagonists is supported by some of our results: the stimulation of the production of PGs by myocytes is also observed under hypoxia, simultaneously with the prevention of the decrease in the glycogen of myocytes.

Despite difficulties of comparing *in vitro* models with clinical situations, the effect of therapeutic concentrations of calcium antagonists on heart cells, under either normal or hypoxic conditions, suggests that calcium antagonists can directly protect myocardium against ischemia and that PGs might play a role in the therapeutic effects of calcium antagonists in coronary heart disease.

Acknowledgements—The authors wish to thank Dr Claude Amiel and Dr Lydie Rappaport for helpful discussions. Authors also thank Knoll Pharmaceutical Co., LERS Synthelabo and Riom CERM Lab. for drug supply, and Dr Jacques Maclouf for providing PGs standards.

REFERENCES

1. K. Schror and S. Moncada, *Prostaglandins* **17**, 367 (1979).
2. S. Alfonso, G. T. Bandow and G. G. Rowe, *J. Physiol.* **241**, 299 (1974).
3. M. L. Ogletree and A. M. Lefer, *Circ. Res.* **42**, 218 (1978).
4. P. R. Lichtlen, H. J. Engel, R. Wolf and I. Amende, in *Calcium Antagonismus* (Eds. A. Fleckenstein and H. Roskamm), p. 270. Springer, Heidelberg (1980).
5. M. B. Pine, P. D. Citron, D. J. Bailly, S. Butman, G. O. Plasencia, D. W. Landa and R. K. Wong, *Circulation* **65**, 17 (1982).
6. I. Yamasawa, Y. Nohara, S. Konno, Y. Nagai, Y. Kanato, H. Takahashi, M. Takanashi, T. Abe, T. Hachiya and H. Sassa, *Drug Res.* **33**, 389 (1983).
7. M. M. Bersohn and K. I. Shine, *J. Mol. Cell. Cardiol.* **15**, 659 (1983).
8. L. B. Rosenberger, L. W. Jacobs and H. C. Stanton, *Life Sci.* **34**, 1379 (1984).
9. L. H. Opie, *Pharm. Ther.* **25**, 371 (1984).
10. M. L. Dahl and P. Uotila, *Prostaglandins Leukotrienes and Med.* **13**, 217 (1984).
11. I. Kai, K. Ogawa and T. Ito, *Jpn Heart J.* **23**, 941 (1982).
12. K. C. Srivastava and K. K. Awasthi, *Prostaglandins Leukotrienes and Med.* **10**, 411 (1983).
13. B. Blondel, I. Roijen and J. P. Cheneval, *Experientia* **27**, 356 (1971).
14. J. L. Samuel, B. Bertier, L. Bugaisky, F. Marotte, B. Swynghedauw, K. Schwartz and L. Rappaport, *Eur. J. Cell. Biol.* **34**, 300 (1984).
15. J. L. Samuel, B. Jockusch, B. Bertier-Savalle, B. Escoubet, F. Marotte, B. Swynghedauw and L. Rappaport, *Bas. Res. Cardiol.* **80**, 99 (1985).
16. L. A. Scharschmidt and M. J. Dunn, *J. clin. Invest.* **71**, 756 (1983).
17. O. H. Lowry, L. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
18. F. Dray, B. Charbonnel and J. Maclouf, *Eur. J. clin. Invest.* **5**, 311 (1975).
19. C. A. Good, H. Kramer and M. Somogyi, *J. biol. Chem.* **100**, 485 (1933).
20. W. W. Seraydarian, I. Harary and E. Sato, *Biochim. biophys. Acta* **162**, 414 (1968).

21. G. G. Ahumada, B. E. Sobel and P. Needleman, *J. Mol. Cell. Cardiol.* **12**, 1287 (1980).
22. H. S. Bolton, R. Ghanderbhan, R. W. Bryant, J. M. Bailey, W. B. Weglicki and G. V. Vahouny, *J. Mol. Cell. Cardiol.* **12**, 1287 (1980).
23. J. Nowak, L. Kaijser and A. Wennmalm, *Prostaglandins and Med.* **4**, 205 (1980).
24. W. G. Brand, J. Nowak and T. Sonnenfeld, *Bas. Res. Cardiol.* **79**, 135 (1984).
25. S. Moncada, *Br. J. Pharmac.* **76**, 3 (1982).
26. M. F. Gerritsen and C. D. Chelic, *J. clin. Invest.* **72**, 1658 (1983).
27. E. A. M. De Decker, D. H. Nugteren and F. T. Hoor, *Nature, Lond.* **268**, 160 (1977).
28. P. D. Henry, *Am. J. Cardiol.* **46**, 1047 (1980).
29. M. D. McGoon, R. E. Ulietstra, D. R. Holmes and J. E. Osborn, *Mayo Clin. Proc.* **57**, 497 (1982).
30. A. Schwartz, I. T. Grupp, G. Grupp, J. S. Williams and P. L. Vaghy, *Biochim. biophys. Res. Commun.* **125**, 387 (1984).
31. G. Thomas, R. Grob and M. Schramm, *J. Cardiovasc. Pharmac.* **6**, 1170 (1984).
32. A. Fleckeistein, *Arzneim. Forsch.* **20**, 1317 (1970).
33. H. Itoh, T. Ishikawa and H. Hidake, *J. Pharmac. exp. Ther.* **230**, 737 (1984).
34. W. G. Nayler, J. S. Dillon and M. J. Daly, in *Calcium Antagonists and Cardiovascular Disease*, p. 181. Raven Press, New York (1984).
35. M. Kolhart, H. P. Haastert and H. Krause, *Pflügers Archs* **342**, 125 (1973).
36. J. Hoerter and L. H. Opie, *Biol. Neonate* **33**, 144 (1978).
37. J. Hoerter, *Pflügers Archs* **363**, 1 (1976).
38. J. Y. Cheung, A. Leaf and J. V. Bonventre, *Am. J. Physiol.* **246**, C323 (1984).
39. H. Araki, *Circ. Res.* **47**, 757 (1980).
40. R. J. Siegel, P. K. Sham, M. Nathen, L. Rodriuez and W. E. Shell, *Am. Heart J.* **108**, 863 (1984).