Br. J. Pharmac. (1980), **69**, 389–396

STUDIES ON THE MECHANISM OF ACTION OF GLUCAGON IN STRIPS OF RABBIT RENAL ARTERY

G. GAGNON, D. REGOLI & F. RIOUX

Department of Physiology and Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4

1 The vasodilator effects of glucagon and adenosine cyclic 3',5'-monophosphate (cyclic AMP) were evaluated in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence and presence of phosphodiesterase inhibitors or calcium (Ca^{2+}) antagonists.

2 The vascular relaxant effect of glucagon was markedly potentiated by various concentrations of four different phosphodiesterase inhibitors (papaverine, theophylline, 3-isobutyl-l-methylxanthine (IBMX) and indomethacin), while that of cyclic AMP was potentiated by only two of them (papaverine and indomethacin) and inhibited by the others (theophylline and IBMX).

3 Amongst the four phosphodiesterase inhibitors, IBMX (10 μg/ml) was found to produce the largest potentiation (e.g. the sensitivity increased by a factor of 10) of glucagon-induced vascular relaxations (ED_{50} of glucagon in the presence of IBMX = 9.2 ± 1.0 ng/ml).

4 Ca^{2+} antagonists such as verapamil and SKF 525A produced a dose-dependent inhibition of the vasodilator action of glucagon. Verapamil (2.5 μg/ml) also antagonized cyclic AMP-induced vascular relaxations.

5 The vasodilator effect of verapamil was inhibited dose-dependently by raising the concentration of extracellular Ca^{2+} from 0.05 to 0.2 g/l (or 1.25 to 5.0 mM) while those elicited by glucagon or cyclic AMP were not influenced, thus suggesting that the latter two drugs do not interfere with Ca^{2+} influx.

6 Disodium edetate (Na_2EDTA, 210 to 840 μg/l) produced a dose-dependent vasodilator effect which was attributed to the facilitation of Ca^{2+} extrusion from the smooth muscle cells and/or Ca^{2+} binding to the cell membrane. The relaxation produced by Na_2EDTA was significantly blocked by verapamil (10 μg/ml) or SKF 525A (10 μg/ml).

7 The results were taken as an indication that glucagon produces at least a fraction of its vasodilator effect by promoting Ca^{2+} extrusion from the vascular smooth muscle cells and/or Ca^{2+} binding to or sequestration into intracellular sites, presumably via a cyclic AMP-dependent mechanism.

Introduction

Besides its well known glycogenolytic and lipolytic activity, glucagon can stimulate the heart or reduce both systemic and regional vascular resistance in animals and in man (for a review, see Kones & Phillips, 1971). During the last decade, numerous studies have appeared describing the vasodilator effects of glucagon in the hepatic (Kock, Roding, Hahnloser, Tibblin & Schenk, Jr., 1970; Richardson & Withrington, 1976 a,b; 1977), mesenteric (Tibblin, Kock & Schenk, Jr., 1970; 1971; Ross, 1970; Ulano, Treat, Shanbour & Jacobson, 1972; MacFerran & Mailman, 1977) or renal (Stowe & Hook, 1970; Levy & Starr, 1972; Levy, 1975 a,b; Ueda, Nakanishi, Miyazaki & Abe, 1977) vascular beds of anaesthetized dogs or cats.

The mechanism by which glucagon induces a decreased vascular resistance in various organs is still unknown. Tibblin *et al.* (1970) obtained results suggesting that the decreased mesenteric vascular resistance provoked by glucagon in anaesthetized dogs was not a consequence of its hyperglycaemic effect. Krarup, Larsen & Munck (1975) subsequently found that the increase in hepatosplanchic blood flow induced by glucagon in cats could be mimicked by a dibutyryl derivative of cyclic 3',5'-monophosphoric acid (cyclic AMP). These observations led to the suggestion that glucagon activates specific vascular receptors and brings about an increased production of cyclic AMP in vascular smooth muscle cells. This hypothesis is consistent with evidence suggesting the involvement of cyclic nucleotides in the process of vascular relaxation (Triner, Nahas, Vulliemoz, Overweg, Verosky, Habif & Ngai, 1971; Andersson, 1972). More recently, we described the direct relaxant effect of glucagon in strips of rabbit renal artery contracted

0007-1188/80/070389-08$01.00

by low concentrations of noradrenaline (Gagnon, Regoli & Rioux, 1978). The effect of glucagon was found resistant to blockade by various drug antagonists and it was attributed to the stimulation of specific receptors. In the same paper, we also described the glucagon potentiating action of papaverine and theophylline, two well known phosphodiesterase inhibitors (Triner *et al.*, 1971).

As an extension of this work, we decided to investigate further the mechanism of the vascular relaxant effect of glucagon using different phosphodiesterase inhibitors and calcium antagonists. Our results are consistent with the view that intracellular cyclic AMP acts as a mediator of the vascular action of glucagon and promotes Ca^{2+} extrusion from the vascular smooth muscle cells and/or Ca^{2+} binding to, or sequestration into, intracellular sites.

Methods

General procedures

The experiments were performed on renal arteries derived from albino rabbits (1.2 to 1.5 kg) of either sex. The animals were killed by a blow on the neck and bled by cutting the carotid arteries. The arteries were taken out, freed from blood and fat and cut helically into strips 2 to 2.5 cm long and 1 to 1.5 mm wide. The tissues were suspended under a resting tension of 1 g in 40 ml organ baths containing a warm (37°C), oxygenated (95% O_2, 5% CO_2) Krebs solution (Gagnon *et al.*, 1978). Changes of tension of the tissues were measured isometrically by force displacement transducers (Grass FT03) coupled to a Grass polygraph (Model 79). The tissues were equilibrated for 90 min before starting the injections of drugs. Volumes of drug injections varied between 0.1 and 0.4 ml.

Construction of dose-response curves

Increasing concentrations of glucagon were applied cumulatively to strips of rabbit renal arteries kept contracted with a low concentration (1 ng/ml) of noradrenaline (NA) which increases the tension of the tissues by 0.8 to 1.0 g. The concentration of NA was increased to different levels (see Table 1) when the tissues were pretreated with different concentrations of papaverine, theophylline, 3-isobutyl-1-methylxanthine (IBMX), indomethacin, verapamil or β-diethylaminoethyldiphenylpropylacetate (SKF 525A) in order to reach the same increase of tension (0.8–1 g) as before. When a stable contraction (plateau) was obtained, the injections of glucagon were repeated. An example of this protocol is given in Figure 1. Two to three dose-response curves to glucagon (1 control and 1 or 2 in the presence of phosphodiesterase inhibitors or calcium antagonists) were obtained in each tissue. Dose-response curves were also performed with cyclic AMP as agonist.

Dose-response curves were also measured with glucagon, cyclic AMP or verapamil on tissues contracted with NA and pre-incubated in a low (0.14 g/l), normal (0.28 g/l) or high (0.56 g/l) calcium (Ca^{2+}) containing Krebs solution. The concentration of Ca^{2+} in the Krebs solution was adjusted to the desired level by adding $CaCl_2$. Disodium ethylenediamine tetraacetate (Na_2EDTA) (210 to 840 μg/l) was used in a few experiments to promote Ca^{2+} efflux from strips of rabbit renal artery contracted with NA in the absence or presence of verapamil or SKF 525A.

Drugs

The following drugs were used: glucagon (Eli Lilly), noradrenaline hydrochloride (NA), papaverine hydrochloride, indomethacin, theophylline, IBMX (Sigma), verapamil hydrochloride (Knoll AG), SKF 525A (Smith, Kline & French), cyclic AMP sodium salt (Sigma). Glucagon was dissolved in the diluting solution for injection provided by Eli Lilly Co. (Gagnon *et al.*, 1978). Indomethacin was dissolved and diluted in trizma base (Sigma) (24.2 g/l). Daily dilutions of the other drugs were made with 0.9% w/v NaCl solution (saline). Concentrations of drugs are expressed in g/ml of the salt with the exception of IBMX, glucagon and indomethacin (g/ml of the base). NA was dissolved in saline acidified with HCl (0.01 N). Ascorbic acid was added to each dilution of NA.

Statistical significance was evaluated by Student's *t* test for paired or independent samples and *P* values of 0.05 or less were considered to be significant.

Results

The effect of phosphodiesterase inhibitors on the vascular relaxant effect of glucagon and cyclic AMP

The vasodilator effects of glucagon or cyclic AMP were tested on strips of rabbit renal artery contracted to produce a tension of 0.8 to 1.0 g with NA (1 ng/ml). In tissues pretreated with papaverine (Ppv), theophylline (Theo), indomethacin (Indo) or IBMX, higher concentrations of NA had to be used to overcome the vasodilator effects exhibited by these drugs and to reach the same tension as elicited with NA (1 ng/ml) (Figure 1). The dose-response curves of glucagon measured in the presence of different concentrations of Ppv, Theo, Indo or IBMX were shifted to the left of the control curve and remained parallel. Figure 2 illustrates the results obtained with glucagon and Ppv. ED_{50} values of glucagon measured in the absence or presence of Ppv, Theo, Indo and IBMX

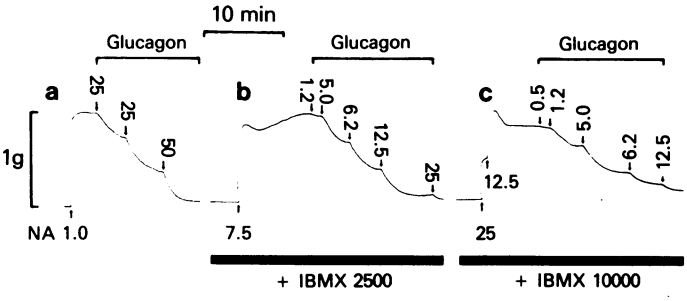

Figure 1 Typical tracings illustrating the vasodilator effects of cumulative applications of glucagon to tissues contracted with noradrenaline (NA) alone (a) or by NA in the presence of 3-isobutyl-1-methylxanthine (IBMX) (b and c). Concentrations of NA, glucagon and IBMX are given in ng/ml.

are summarized in Table 1. IBMX appears to potentiate glucagon to a larger extent than the other drugs. High concentrations of Ppv (μg/ml) or Indo (200 μg/ml) lose their ability to potentiate the vasodilator effect of glucagon.

The vascular relaxant effect of exogenous cyclic AMP was potentiated by Ppv and Indo but it was markedly depressed by IBMX and Theo. The dose-response curves obtained with cyclic AMP in the absence and presence of Ppv (2.5 μg/ml) are shown in Figure 3. The ED_{50} values of cyclic AMP measured in tissues exposed to NA alone or to NA in the presence of phosphodiesterase inhibitors are summarized in Table 2. The concentrations of phosphodiesterase inhibitors used in the cyclic AMP experiments were the same as those found to produce the maximum potentiating effect of glucagon (see Table 1). On the basis of ED_{50} values, it appears that the vasodilator

Table 1 ED_{50} values of glucagon determined on strips of rabbit renal artery contracted with noradrenaline (NA) in the absence or presence of phosphodiesterase inhibitors or calcium antagonists[a]

Drug treatment (g/ml)			*ED_{50} for glucagon* (ng/ml)
NA	(1.0×10^{-9})		86.5 ± 1.9 (85)
NA	(5.0×10^{-9})	plus papaverine	(1.0×10^{-6}) 61.2 ± 5.6 (11)*
NA	(1.5×10^{-8})	plus papaverine	(2.5×10^{-6}) 29.2 ± 2.0 (9)*
NA	(5.0×10^{-7})	plus papaverine	(1.0×10^{-5}) 78.7 ± 9.5 (9) NS
NA	(5.0×10^{-8})	plus theophylline	(2.5×10^{-4}) 62.7 ± 5.7 (8)*
NA	(2.5×10^{-7})	plus theophylline	(1.0×10^{-3}) 32.2 ± 4.2 (8)*
NA	(2.5×10^{-8})	plus indomethacin	(1.0×10^{-5}) 72.0 ± 2.3 (6)*
NA	(1.2×10^{-7})	plus indomethacin	(5.0×10^{-5}) 38.3 ± 3.6 (6)*
NA	(2.5×10^{-7})	plus indomethacin	(2.0×10^{-4}) 67.0 ± 6.3 (6)*
NA	(1.0×10^{-8})	plus IBMX	(1.0×10^{-6}) 40.5 ± 3.1 (4)*
NA	(1.5×10^{-8})	plus IBMX	(2.5×10^{-6}) 31.7 ± 4.6 (9)*
NA	(3.5×10^{-8})	plus IBMX	(1.0×10^{-5}) 9.2 ± 1.0 (9)*
NA	(1.2×10^{-8})	plus verapamil	(2.5×10^{-7}) 228.0 ± 27.0 (7)*
NA	(4.0×10^{-8})	plus verapamil	(1.0×10^{-6}) 375.0 ± 50.0 (4)*
NA	(7.5×10^{-8})	plus verapamil	(2.5×10^{-6}) 395.6 ± 18.6 (8)*
NA	(2.5×10^{-7})	plus verapamil	(1.0×10^{-5}) 438.0 ± 64.0 (4)*
NA	(2.0×10^{-9})	plus SKF525A	(1.0×10^{-6}) 127.5 ± 5.2 (4)*
NA	(3.5×10^{-9})	plus SKF525A	(2.5×10^{-6}) 251.0 ± 35.6 (6)*
NA	(2.5×10^{-8})	plus SKF525A	(5.0×10^{-6}) 508.0 ± 66.0 (6)*
NA	(4.5×10^{-7})	plus SKF525A	(1.0×10^{-5}) 983.3 ± 115.0 (6)*

[a] The statistical significance was calculated by comparing the ED_{50} value of glucagon measured in the presence of NA alone with those obtained in the presence of NA and other drugs. In parentheses, the number of individual determinations. The results are expressed as means ± s.e. mean.
* $P < 0.001$

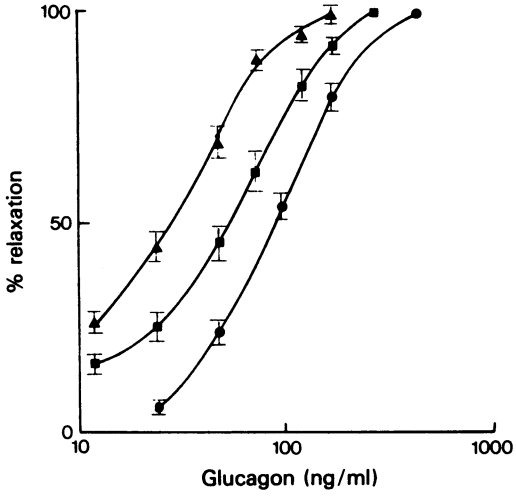

Figure 2 Dose-response curves to glucagon measured in strips of rabbit renal artery contracted with noradrenaline in the absence (●) or presence of papaverine 1 (■) and 2.5 μg/ml (▲). Each point is the mean ± s.e. means of 9–11 individual determinations. The various concentrations of NA used to contract the tissues by 0.8–1.0 g in the presence of PPV are given in Table 1.

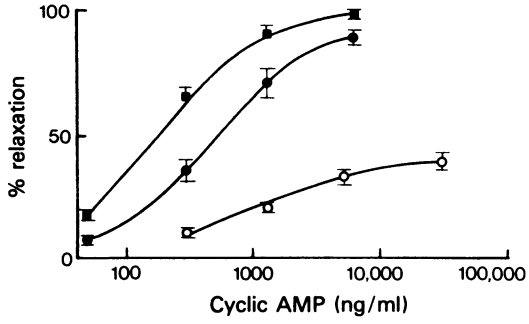

Figure 3 Dose-response curves to cyclic AMP measured in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence (●) or presence of papaverine, 2.5 μg/ml (■) or verapamil, 2.5 μg/ml (○). Each point is the mean of 8–10 individual determinations; vertical lines show s.e. mean. The various concentrations of NA used to contract the tissues to give a tension of 0.8–1.0 g in the presence of papaverine or verapamil are given in Table 2.

effects of cyclic AMP are potentiated to a similar extent to those produced by glucagon, by the presence in the organ bath of Ppv (2.5 μg/ml) or Indo (50 μg/ml). The large inhibition of cyclic AMP-induced vascular relaxations by IBMX and Theo will be discussed below.

The inhibition of the vascular effect of glucagon and cyclic AMP by calcium antagonists

Ca^{2+} antagonists such as verapamil (Vrpm) or SKF 525A (Kalsner, Nickerson & Boyd, 1979) inhibited dose-dependently the vasodilator effect of glucagon in strips of rabbit renal artery. The dose-response curves of glucagon were shifted to the right and remained parallel in the presence of Vrpm (0.25 and 2.5 μg/ml) (Figure 4) or SKF 525A (2.5 and 5.0 μg/ml). However, the curve became flattened and exhibited a depressed maximum in the presence of SKF 525A, 10 μg/ml. The two Ca^{2+} antagonists increased significantly the ED_{50} values of glucagon (Table 1).

Vrpm was also tested against cyclic AMP. The dose-response curve of cyclic AMP obtained in the presence of Vrpm (2.5 μg/ml) was displaced to the right of the control curve and showed a reduced maximum effect. This result is illustrated in Figure 3. The ED_{50} value of cyclic AMP measured in the presence of Vrpm is given in Table 2.

Table 2 ED_{50} values of cyclic AMP determined on strips of rabbit renal artery contracted with noradrenaline (NA) in the absence or presence of phosphodiesterase inhibitors or verapamil[a]

	Drug treatment (g/ml)		*ED_{50} for cyclic AMP* (ng/ml)
NA	(1.0×10^{-9})		693.7 ± 66 (32)
NA	(1.5×10^{-8})	plus papaverine (2.5×10^{-6})	206.3 ± 36.2 (10)**
NA	(3.5×10^{-8})	plus IBMX (1.0×10^{-5})	4612.5 ± 494.8 (8)**
NA	(2.5×10^{-7})	plus theophylline (1.0×10^{-3})	NM[b] (8)
NA	(1.2×10^{-8})	plus indomethacin (5.0×10^{-5})	233.7 ± 33.3 (8)**
NA	(7.5×10^{-8})	plus verapamil (2.5×10^{-6})	1122.5 ± 146.4 (8)*

[a] The statistical significances were calculated by comparing the ED_{50} value of cyclic AMP measured in the presence of NA alone with those obtained in the presence of NA and other drugs. In parentheses, the number of individual determinations. The results are expressed as means ± s.e. mean.

* $P < 0.02$; ** $P = 0.001$.

[b] NM = not measurable; theophylline $(1 \times 10^{-3}$ g/ml) blocked completely the relaxant effect of cyclic AMP.

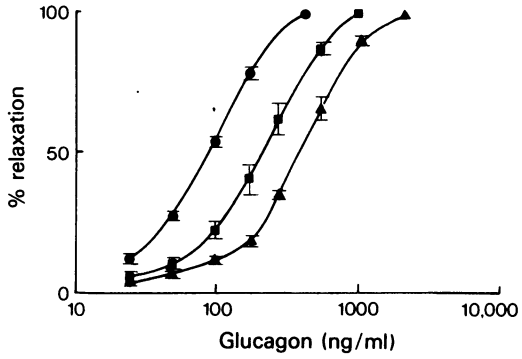

Figure 4 Dose-response curves to glucagon as measured in strips of rabbit renal artery contracted with noradrenaline (NA) in the absence (●) or presence of verapamil 0.25 (■) and 2.5 μg/ml (▲). Each point is the mean of 4–8 individual determinations; vertical lines show s.e. means. The various concentrations of NA used to contract the tissues by 0.8–1.0 g in the presence of verapamil are given in Table 1.

The influence of various extracellular Ca^{2+} concentrations upon the vasodilator effects of glucagon, cyclic AMP and verapamil

These experiments were designed to evaluate the possibility that glucagon and cyclic AMP produced their vascular relaxant effect by interfering with the entry of Ca^{2+} into the vascular smooth muscle cells. Vrpm, a well known inhibitor of Ca^{2+} influx into myocardial and smooth muscle cells (Fleckenstein, 1977; Andersson, 1978), induced a dose-dependent relaxation of strips of rabbit renal artery contracted with NA. The ED_{50} values of Vrpm measured in a low (0.05 g/l) Ca^{2+}-containing medium increased significantly when determined in normal (0.1 g/l) or high (0.2 g/l) Ca^{2+}-containing media thus suggesting that Ca^{2+} can reverse the blockade of Ca^{2+} influx by Vrpm (Table 3). In contrast, the vasodilator effects of glucagon and cyclic AMP were not affected by varying the concentration of extracellular Ca^{2+}.

In a few experiments, Na_2EDTA, a metal chelator, was applied in a concentration varying between 210 and 840 μg/l to strips of rabbit renal artery contracted with NA or NA plus Vrpm (10 μg/ml) or SKF 525A (10 μg/ml). In the absence of Vrpm or SKF 525A, Na_2EDTA induced a rapid dose-dependent vasodilator effect (Figure 5). The latter effect was significantly reduced in the presence of Vrpm or SKF 525A. The vasodilator effect of Na_2EDTA was also significantly inhibited by Vrpm (250 and 2500 ng/ml). Vrpm (2500 ng/ml) also inhibits (G. Gagnon, D. Regoli F. Rioux, unpublished results) the vasodilator effect of ethyleneglycol-bis (β-aminoethyl ether) N,N'-tetraacetic acid (EGTA), a more specific Ca^{2+} chelator.

Discussion

Previous work from this laboratory led to the suggestion that the vasodilator effect of glucagon in strips of rabbit renal artery was mediated by the activation of a vascular adenylate cyclase; this process leading to the accumulation of cyclic AMP inside the smooth muscle cells (Gagnon *et al.*, 1978). This interpretation was based on the following observations: (1) phosphodiesterase inhibitors such as Ppv and Theo, potentiated the vascular relaxant action of glucagon; (2) exogenously applied cyclic AMP mimicked the effect of glucagon. In the present paper, we confirmed these observations. Furthermore, we have shown that IBMX, a very potent and widely used phosphodiesterase inhibitor (Wells, Wu, Baird & Hardman, 1975) potentiated to a greater extent than Ppv the vascular effect of glucagon (Table 1). The potentiation of glucagon by Indo, a well known inhibitor of prostaglandin synthesis (Vane, 1971) may also be explained by the ability of this drug to inhibit phosphodiesterases (Flores & Sharp, 1972; Newcombe, Thanassi & Ciosek Jr., 1974). Since phosphodiesterase inhibitors

Table 3 ED_{50} values of glucagon, cyclic AMP and verapamil as determined in strips of rabbit renal artery contracted with noradrenaline (1.5 ng/ml) in the presence of different extracellular Ca^{2+} concentrations[a]

Extracell. Ca^{2+} (g/l)	ED_{50} (ng/ml) Glucagon	Cyclic AMP	Verapamil
0.02	80.7 ± 6.6	—	—
0.05	80.7 ± 3.7	696.8 ± 68.4	28.6 ± 2.5**
0.1	82.8 ± 5.9	702.5 ± 70.8	95.0 ± 13.3
0.2	78.2 ± 6.3	665.0 ± 68.6	241.0 ± 36.9*

[a] The results are expressed as means ±s.e. means of 8 individual determinations. The statistical significances were evaluated by comparing the ED_{50} values obtained in different Ca^{2+} containing media with those determined in the presence of 0.1 g/l or 2.5 mM Ca^{2+} concentration (control medium) for each compound, respectively.
* $P < 0.005$; ** $P < 0.001$.

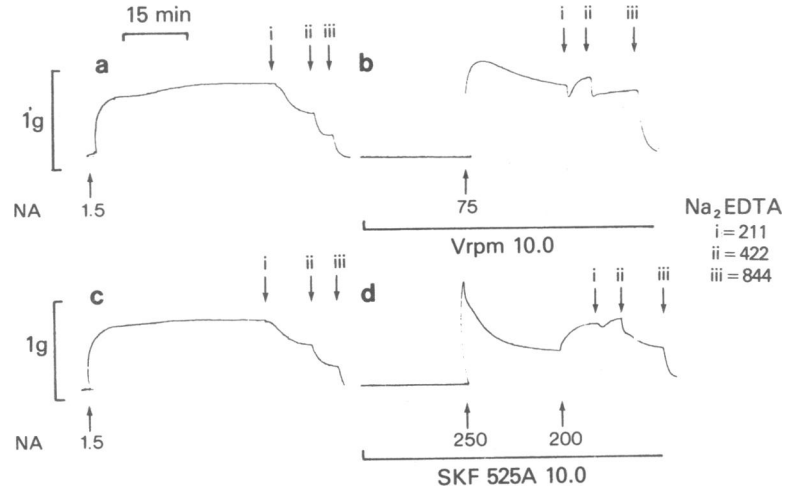

Figure 5 Typical tracings illustrating the vasodilator effects of cumulative applications of disodium edetate (Na_2EDTA) to tissues contracted with noradrenaline (NA) in the absence (a and c) or presence of verapamil (Vrpm) (b) or SKF 525A (d). Concentrations of NA are in ng/ml while those of Vrpm and SKF 525A are in μg/ml and those of Na_2EDTA are in μg/l.

are known to favour the intracellular accumulation of cyclic AMP in various organs including vascular tissues (Triner *et al.*, 1971; Andersson, 1972) by blocking the enzymatic conversion of cyclic AMP to 5'-AMP, they may also potentiate the vascular relaxant effect of glucagon by such a mechanism. If this interpretation is correct, we would expect the vasodilator effect of exogenous cyclic AMP to be affected in a similar way to glucagon by these drugs. Indeed, Ppv and Indo potentiated the effect of cyclic AMP (Table 2). In contrast, IBMX and Theo markedly inhibited cyclic AMP-induced vascular effects. The ability of methylxanthines to inhibit the action of exogenous nucleotides (e.g. ATP, ADP, AMP, adenosine or cyclic AMP) has been described in other systems (Sawynock & Jhamandas, 1976; Verhaeghe, Vanhoutte & Shepherd, 1977; Wakade & Wakade, 1978) and attributed in most cases to the blockade of common adenosine receptors presumably located in the cell membranes. This hypothesis may explain the observed inhibition of cyclic AMP-induced vascular relaxation by Theo and IBMX (Table 2). Furthermore, it may suggest that the sites of action of extracellular cyclic AMP may be different from those on which intracellularly produced cyclic AMP (for instance, following the action of glucagon) may be acting. This interpretation is consistent with the observed potentiation of glucagon-induced vascular relaxation by four different phosphodiesterase inhibitors and the well known property of glucagon of increasing the intracellular cyclic AMP levels in several organs such as liver, adipose tissues and hearts (Sutherland, Robison & Butcher, 1968). Our results are also consistent with a hypothetical model in which intracellular and extracellular receptor sites of cyclic AMP would subserve a similar function (e.g. to contribute to a reduction of intracellular free Ca^{2+}). The absence of inhibition of cyclic AMP by Ppv and Indo may be due to the fact that, being chemically less closely related to the adenosine moiety of cyclic AMP than methylxanthines, they cannot compete with cyclic AMP for its receptor sites. Assuming that cyclic AMP can produce its vascular relaxant action without entering the cells, its potentiation by Ppv and Indo raised the possibility that phosphodiesterase located in cell membranes may be accessible to extracellular cyclic AMP in vascular tissues. Such an interpretation is consistent with previous reports on the subcellular distribution of these enzymes in various organs (De Robertis, De Lores Arnaiz, Alberici, Butcher & Sutherland, 1967; Cheung, 1967).

The antagonism of glucagon- or cyclic AMP-induced vascular relaxation by Ca^{2+} antagonists (Vrpm, SKF 525A) deserves further elaboration. The ability of Vrpm or SKF 525A to inhibit cardiac or vascular smooth muscle contractions induced by some agonists has been emphasized by various authors (for a review, see Fleckenstein, 1977; and Andersson, 1978). These drugs are believed to interfere with the entry of Ca^{2+} into the cells. Their inhibitory action is reversed by increasing the concentration of extracellular Ca^{2+} (Fleckenstein, 1977). Having this in mind, we were at first tempted to propose that glucagon or exogenous cyclic AMP produce part of their vascular relaxant effects by acting on the same receptor sites as Vrpm or SKF 525A. This interpreta-

tion had to be rejected following our observation that an increase in the extracellular Ca^{2+} concentration did not influence the vasodilator effects of glucagon and cyclic AMP but markedly reduced that elicited by Vrpm (Table 3). The possibility that glucagon and exogenous cyclic AMP produced their action by facilitating Ca^{2+} extrusion from the vascular smooth muscle cells and/or Ca^{2+} uptake or binding to cellular Ca^{2+}-binding sites (e.g. endoplasmic reticulum, plasma membranes, mitochondria) was then considered and is now the interpretation that we favour the most to explain the inhibition of the vascular relaxant effect of glucagon and cyclic AMP by Ca^{2+} antagonists. The assumption is that Ca^{2+} antagonists such as Vrpm or SKF 525A can interfere also with Ca^{2+} extrusion from the smooth muscle cells and/or Ca^{2+} binding to cellular Ca^{2+} binding sites. The blockade of Na_2EDTA-induced vascular relaxation (Figure 5) by Vrpm and SKF 525A gives further support to our hypothesis since the mechanism whereby Na_2EDTA induced its vascular relaxant effect is likely to involve both a facilitation of Ca^{2+} extrusion from the smooth muscle cells and/or Ca^{2+} binding to cell membranes. Whether or not Vrpm and SKF 525A have the ability to enter the cells and to interfere with the process of Ca^{2+} binding to and/or sequestration into intracellular elements is still unknown. Further studies are obviously needed before definite conclusions can be drawn on the mechanism whereby Vrpm and SKF 525A inhibit drug-induced vascular relaxations.

The pharmacological evidence reported above suggests that glucagon may produce its vasodilator effect in strips of rabbit renal artery contracted by NA, by 'turning on' the intracellular cyclic AMP-generating system of the smooth muscle cells. According to various authorities (Andersson, 1972; 1978; Rasmussen, 1976), intracellular cyclic AMP contributes to smooth muscle relaxation by promoting the binding of Ca^{2+} to intracellular membranes (e.g. endoplasmic reticulum) and its reuptake in mitochondria, and by accelerating the process of Ca^{2+} extrusion or efflux from the cells. Our experiments with Ca^{2+} antagonists suggest that both extracellular or intracellular cyclic AMP may bring about relaxation of vascular tissues by promoting Ca^{2+} extrusion from the smooth muscle cells and/or Ca^{2+} binding to cellular Ca^{2+}-binding sites. Further studies are needed to support this hypothesis.

A technical point needs also to be discussed briefly. In the presence of phosphodiesterase inhibitors or Ca^{2+} antagonists, the concentration of NA required to elicit 0.8 to 1.0 g of tension had to be increased since these drugs are vasodilators and relax the renal artery contracted with NA. Their effect was overcome and eliminated simply by increasing the concentration of the vasoconstrictor (e.g. physiological antagonism).

In conclusion, we would like to underline two important consequences of this work: (1) it has been possible to improve greatly the sensitivity of our previously described bioassay for glucagon by the use of phosphodiesterase inhibitors such as IBMX (Gagnon *et al.*, 1978); (2) the pharmacological approach described in this paper may be of great value in distinguishing vasodilators which act by cyclic AMP-dependent- or cyclic AMP-independent mechanisms.

We wish to express our thanks to Dr R. S. Dolman from Eli Lilly Co., Toronto, Ontario for the generous gift of glucagon. We also thank Mrs Diane Tousignant for typing the manuscript. This work was supported by grants from the Canadian Heart Foundation (CHF), the Medical Research Council of Canada (MRCC) and the Banting Research Foundation. D.R. is an associate of the MRCC. F.R. is a scholar of the CHF and G.G. has a studentship from the Ministère de l'Education du Québec. Reprint requests to F.R.

References

ANDERSSON, R.G.G. (1972). Cyclic AMP and calcium ions in mechanical and metabolic responses of smooth muscles; influence of some hormones and drugs. *Acta physiol. scand.*, suppl. **382**, 1–59.

ANDERSSON, K.E. (1978). Effects of calcium and calcium antagonists on the excitation-contraction coupling in striated and smooth muscle. *Acta pharmac. tox.*, **43**, suppl. 1, 5–14.

CHUNG, W.Y. (1967). Properties of cyclic 3',5'-nucleotide phosphodiesterase from rat brain. *Biochemistry*, **6**, 1079–1087.

DE ROBERTIS, E., DE LORES ARNAIZ, G.R., ALBERICI, M., BUTCHER, R.W. & SUTHERLAND, E.W. (1967). Subcellular distribution of adenyl cyclase and cyclic phosphodiesterase in rat brain cortex. *J. biol. Chem.*, **242**, 3487–3493.

FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *A. Rev. Pharmac. tox.*, **17**, 149–166.

FLORES, A.G.A. & SHARP, G.W.G. (1972). Endogenous prostaglandins and osmotic water flow in the toad bladder. *Am. J. Physiol.*, **233**, 1392–1397.

GAGNON, G., REGOLI, D. & RIOUX, F. (1978). A new bioassay for glucagon. *Br. J. Pharmac.*, **64**, 99–108.

KALSNER, S., NICKERSON, M., & BOYD, G.N. (1970). Selective blockade of potassium-induced contractions of aortic strips by β-diethylamino-ethyldiphenylpropyl-acetate (SKF 525A). *J. Pharmac. exp. Ther.*, **174**, 500–508.

KOCK, N.G., RODING, B., HAHNLOSER, P., TIBBLIN, S. & SCHENK JR., W.G. (1970). The effect of glucagon on hepatic blood blow. *Arch. Surg.*, **100**, 147–149.

KONES, R.J. & PHILLIPS, J.H. (1971). Glucagon: present

status in cardiovascular disease. *Clin. Pharmac. Ther.*, **12**, 427–444.

KRARUP, N., LARSEN, J.A. & MUNCK, A. (1975). Imitation of glucagon effects on splanchnic hemodynamics and liver function by N^6,2'-O-dibutyryl 3',5' cyclic AMP (DBc AMP) in cats. *Acta physiol. scand.*, **95**, 110–116.

LEVY, M. (1975a). The effect of glucagon on glomerular filtration rate in dogs during reduction of renal blood flow. *Can. J. Physiol. Pharmac.*, **53**, 660–668.

LEVY, M. (1975b). Further observations on the response of the glomerular filtration rate to glucagon: comparison with secretin. *Can. J. Physiol., Pharmac.*, **53**, 81–85.

LEVY, M. & STARR, N.L. (1972). The mechanism of glucagon-induced natriuresis in dogs. *Kidney Int.*, **2**, 76–84.

MACFERRAN, S.N. & MAILMAN, D. (1977). Effects of glucagon on canine intestinal sodium and water fluxes and regional blood flow. *J. Physiol.*, **266**, 1–12.

NEWCOMBE, D.S., THANASSI, N.M. & CROSEK JR., C.P. (1974). Cartilage cyclic nucleotide phosphodiesterase: inhibition by anti-inflammatory agents. *Life Sci., Oxford*, **14**, 505–519.

RASMUSSEN, H. (1976). Ions as second messengers. In *Cell Membranes, Biochemistry, Cell Biology & Pathology*. ed. Weissman, G. & Claiborne R. pp. 203–212. New York: H.P. Publishing Co.

RICHARDSON, P.D.I. & WITHRINGTON, P.G. (1976a). The inhibition by glucagon of the vasoconstrictor actions of noradrenaline, angiotensin and vasopressin on the hepatic arterial vascular bed of the dog. *Br. J. Pharmac.*, **57**, 93–102.

RICHARDSON, P.D.I. & WITHRINGTON, P.G. (1976b) The vasodilator actions of isoprenaline, histamine, prostaglandin E_2, glucagon and secretin on the hepatic arterial vascular bed of the dog. *Br. J. Pharmac.*, **57**, 581–588.

RICHARDSON, P.D.I., & WITHRINGTON, P.G. (1977). Glucagon, inhibition of hepatic arterial responses to hepatic nerve stimulation. *Am. J. Physiol.*, **233**, 647–654.

ROSS, G. (1970). Regional circulatory effects of pancreatic glucagon. *Br. J. Pharmac.*, **38**, 735–742.

SAWYNOK, J. & JHAMANDAS, K.H. (1976). Inhibition of acetylcholine release from cholinergic nerves by adenosine, adenine nucleotides and morphine: antagonism by theophylline. *J. Pharmac. exp. Ther.*, **197**, 379–390.

STOWE, N.T. & HOOK, J.B. (1970). Role of alterations in renal hemodynamics in the natriuretic action of glucagon. *Archs. int. Pharmacodyn. Thér.*, **183**, 65–74.

SUTHERLAND, E.W., ROBINSON, G.A. & BUTCHER, R.W. (1968). Some aspects of the biological role of adenosine 3',5'-monophosphate (Cyclic AMP). *Circulation Res.*, **37**, 279–306.

TIBBLIN, S., KOCK, N.G. & SCHENK JR., W.G. (1970). Dissociation of the hyperglycemic and vascular effects of glucagon. *Surgery*, **67**, 816–825.

TIBBLIN, S., KOCK, N.G. & SCHENK JR., W.G. (1971). Response of mesenteric blood flow to glucagon. *Arch. Surg.*, **102**, 65–70.

TRINER, L., NAHAS, G.G., VULLIEMOZ, Y., OVERWEG, N.I.A., VEROSKY, M., HABIF, D.V. & NGAI, S.H. (1971). Cyclic AMP and smooth muscle function. *Ann. N.Y. Acad. Sci.*, **185**, 458–476.

UEDA, J., NAKANISHI, H., MIYAZAKI, M. & ABE, Y. (1977). Effects of glucagon on the renal hemodynamics of dogs. *Eur. J. Pharmac.*, **41**, 209–212.

ULANO, H.B., TREAT, E., SHANBOUR, L. L., & JACOBSON, E.D. (1972). Selective dilatation of the constricted superior mesenteric artery. *Gastroenterology*, **62**, 39–47.

VANE J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 231–235.

VERHAEGHE, R.H., VANHOUTTE, P.M. & SHEPHERD, J.T. (1977). Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides. *Circulation Res.*, **40**, 208–215.

WAKADE, A.R., & WAKADE, T.D. (1978). Inhibition of noradrenaline release by adenosine. *J. Physiol.*, **282**, 35–49.

WELLS, J.N., WU, Y.J., BAIRD, C.E. & HARDMAN, J.G. (1975). Phosphodiesterases from porcine coronary arteries: inhibition of separated forms by xanthine, papaverine and cyclic nucleotides. *Mol. Pharmac.*, **11**, 775–783.

(Received March 12, 1979. Revised September 28, 1979.)