Bhattacharya, S. K., Das, N. (1985a) Pharm. Res. in press
Bhattacharya, S. K., Das, N. (1985b) Agents Actions, in press
Bonta, I. L. (1978) in: Vane, J. R., Ferreira, S. H. (eds) Handbook of Experimental Pharmacology, Vol. 50/I. Springer-Verglag, New York, pp 523–567
Das, N., Bhattacharya, S. K. (1983) Ind. J. Pharmacol. 15: 64–65.
Das, N., Bhattacharya, S. K. (1985) Pharm. Res. 3: 137–139
Enna, S. J. (1979) Annu. Rep. Med. Chem. 14: 41–50
Fearn, H. J., Karady, S., West, G. B. (1965) J. Pharm. Pharmacol. 17: 761–765
Feldberg, W., Lotti, V. J. (1967) Br. J. Pharmacol. Chemother. 31: 152–161
Horrobin, D. F. (1977) Lancet i: 936–937
Iversen, L. L. (1978) in: Lipton, M. A., DiMascio, A., Killam, K. F. (eds) Psychopharmacology: A generation of progress. Raven Press, New York, pp 25–38
Lombardino, J. G., Otterness, K. G., Wiseman, E. H. (1975) Arzneimittel-Forsch. 25: 1629–1635
Pradhan, S. N., Bose, S. (1978) in: Lipton, M. A., DiMascio, A., Killam, K. F. (eds) Psychopharmacology: A generation of progress. Raven Press, New York, pp 271–281
Winter, C. A., Risely, E. A., Nuss, G. W. (1962) Proc. Soc. Exp. Biol. 111: 544–547

J. Pharm. Pharmacol. 1986, 38: 146–149
Communicated July 22, 1985

Effect of some vasodilators on cat femoral arteries

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Adenosine, cyclic-AMP (cAMP), papaverine and 1-methyl-3-isobutylxanthine (MIX) evoked dose-dependent vasodilatation in cat femoral arteries precontracted with 75 mM K^+. The vasodilator response induced at maximal concentration used was: papaverine > MIX > adenosine = cAMP. With regard to the potency of relaxant effects (IC10) the order was MIX = cAMP = papaverine > adenosine. The dilatation elicited by papaverine, adenosine and cAMP was increased by MIX. Preincubation with adenosine enhanced the relaxation induced by MIX and reduced that produced by cAMP. These results indicate that the effects of adenosine and cAMP seem not to be mediated by specific surface receptors but by a cAMP-dependent mechanism. The interference between adenosine and cAMP could be due to competition for a similar site and/or mechanism.

It has been suggested that the intracellular increase in cyclic-AMP (cAMP) is the mechanism by which some drugs produce relaxation of smooth muscle (Triner et al 1971; Andersson 1972; Gagnon et al 1980b). This compound seems to interfere with Ca^{2+} movements and free Ca^{2+} levels in the cells (Gagnon et al 1980b). There are several agents that increase the amount of cAMP by phosphodiesterase inhibition or activation of adenylate cyclase. In the former group are papaverine and xanthines (Triner et al 1971; Wells et al 1975) and the latter could include adenosine and related compounds (Kukovetz et al 1978; Collis & Brown 1983; Edvinsson & Fredholm 1983). Furthermore, the xanthines also have the ability to block the purinoreceptors located on the cell surfaces, antagonizing the effects of adenosine and analogous compounds (Gagnon et al 1980b; Toda et al 1982; Edvinsson & Fredholm 1983).

Papaverine (Toda 1974; Gagnon et al 1980b), xanthines (Gagnon et al 1980b) and adenosine and related compounds (Napoli et al 1980; Gagnon et al 1980a,b; Toda et al 1982) induce vasodilator responses in different vessels, probably as a consequence of the mechanisms of action described above.

The aim of the present study was to analyse the probable vasodilator effects of adenosine, cAMP, papaverine and 1-methyl-3-isobutylxanthine (MIX) as well as the possible interaction between them in cat femoral arteries.

Materials and methods

Cats (1·5–3 kg) were anaesthetized by i.p. administration of 35 mg kg^{-1} of sodium pentobarbitone and killed by bleeding. The femoral arteries were removed and dissected into cylindrical segments 4 mm in length. Each arterial cylinder was set up for isometric recording in an organ bath, as described by Nielsen & Owman (1971), containing 6 ml of Krebs-Henseleit solution (KHS) at 37 °C continuously bubbled with a 95% O_2–5% CO_2 mixture, which gave a pH of 7·4–7·5. Two stainless steel pins were introduced through the lumen of the arterial segments. One pin was fixed to the organ bath wall, while the other one was connected to a strain gauge for isometric tension recording. The latter pin was parallel with the former and was movable, thus permitting the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder. The isometric contraction was recorded through a force-displacement transducer (Grass FTO3C) connected to a Grass Model 7D Polygraph. A resting tension of 1 g was applied to the segments which were readjusted every 15 min during a 90–120 min equilibration period before cumulative dose–response curves for different drugs were performed. The composition of the KHS was (mM): NaCl 115; KCl 4·6; $CaCl_2$ 2·5; KH_2PO_4 1·2; $MgSO_4.7H_2O$ 1·2; $NaHCO_3$ 25; glucose 11·1 and the disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) 0·03.

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To analyse the vasodilator effects of the drugs used in this study the arterial segments were given an active tone with 75 mM KCl and when the contraction reached a plateau the dose–response curve for each drug was carried out cumulatively. The vessels were then washed several times, and on baseline recovery the curve was repeated although now the arteries had a previous 20 min exposure to the corresponding drug for interfering with relaxation. When MIX or adenosine was used for this purpose a reduction of resting tone normally did not appear. The contraction elicited by K^+ was either unaffected or slightly decreased by MIX. Since papaverine did not disappear on washing, the arteries exposed to this agent were used only once. Drugs were dissolved in physiological saline solution containing 0·01% (w/v) ascorbic acid, except MIX which was dissolved in slightly alkaline distilled water.

The drugs used were adenosine, cyclic(c)AMP sodium salt, 1-methyl-3-isobutylxanthine and papaverine hydrochloride obtained from Sigma. The relaxant responses evoked by these agents were calculated as percentage of inhibition of K^+ (75 mM)-induced contractions. The concentrations of drugs eliciting 10% relaxation (IC10) were used when comparing potency, since this response was near the maximum vasodilation obtained by adenosine and cAMP. The drug dose–response curves did not reach a maximum. The IC10 was calculated according to the method of Fleming et al (1972). Results shown in the figures and text represent the mean values ± s.e.m. Statistical significance was determined by Student's *t*-test; a probability value of less than 5% was considered significant.

Results

The vasodilatation induced by papaverine in segments of femoral arteries previously exposed to 75 mM K^+ is shown in Fig. 1. Preincubation for 20 min with $5·5 \times 10^{-5}$ M MIX increased the relaxation, which was only significant at 10^{-5} and 3×10^{-5} M papaverine. The concentration of this xanthine did not usually affect the contraction elicited by K^+. Adenosine also produced dose-dependent vasodilatation under the same conditions. Pre-addition to the bath of the same concentration of MIX increased the depressor response caused by adenosine (Fig. 2). MIX elicited relaxant effects, which were enhanced by previous exposure of the segments with 5×10^{-5} M adenosine (Fig. 3), which did not change the contractile response evoked by K^+. cAMP produced a dose-dependent vasodilatation which was markedly reduced in the presence of 5×10^{-5} M adenosine and increased by pre-addition of $5·5 \times 10^{-5}$ M MIX to the bath (Fig. 4).

The relaxation obtained at the maximal concentration of the drugs used and IC10 are shown in Table 1. The maximal response was: papaverine > MIX > adenosine = cAMP, and with regard to the potency to induce vasodilatation (IC10): MIX = cAMP = papaverine > adenosine.

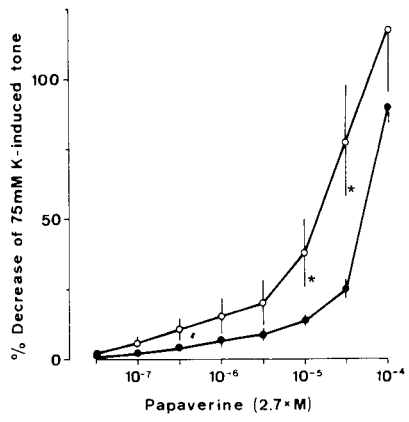

FIG. 1. Effect of $5·5 \times 10^{-5}$ M (MIX) on the dose–response curve to papaverine on femoral artery segments previously contracted with 75 mM K^+. Mean ± s.e.m. isometric responses for K^+ was of 1750 ± 210 mg. Each point represents the mean ± s.e.m. * $P < 0·05$. ● Control (8), ○ MIX (7). (No. of arterial segments.)

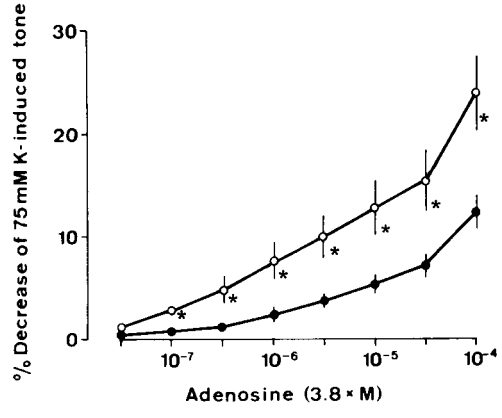

FIG. 2. Effect of $5·5 \times 10^{-5}$ MIX on the dose–response curve to adenosine. *$P < 0·05$. ● Control (5), ○ MIX (6).

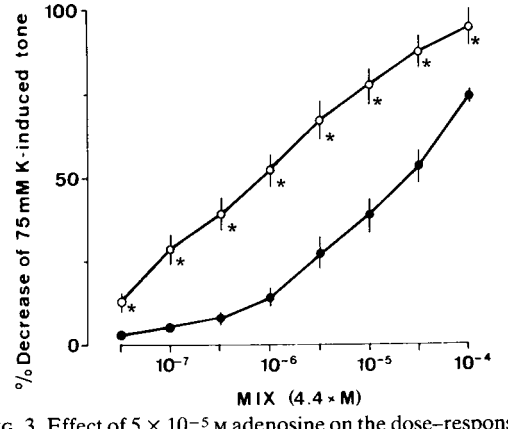

FIG. 3. Effect of 5×10^{-5} M adenosine on the dose–response curve to MIX. *$P < 0·05$. ● Control (14), ○ adenosine (7).

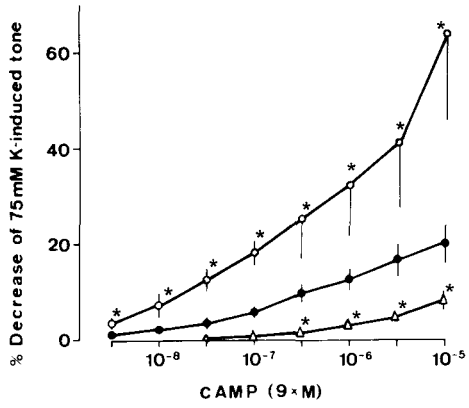

FIG. 4. Effect of 5×10^{-5} M adenosine or $5{\cdot}5 \times 10^{-5}$ M MIX on the dose–response curve to cAMP on segments of femoral arteries previously contracted with 75 mM K^+. *$P < 0{\cdot}05$. ● Control (14), ○ MIX (5), △ adenosine (6).

Table 1. Vasodilator responses obtained at the maximal concentration of the drugs used (C_{max}) and IC10 (8 to 17 femoral artery segments were used in each case).

	E_{max} + s.e.m. (mg)	C_{max} (M)	IC10 (M) (95% confidence interval)
Papaverine	1695 ± 199	2×10^{-4}	$8{\cdot}5 \times 10^{-6}$ (1·8–39)
MIX	1125 ± 271	$4{\cdot}4 \times 10^{-4}$	$2{\cdot}8 \times 10^{-6}$ (0·9–8·7)
Adenosine	271 ± 58	$3{\cdot}8 \times 10^{-4}$	$2{\cdot}1 \times 10^{-4}$ (0·6–7)
cAMP	294 ± 81	9×10^{-5}	$8{\cdot}0 \times 10^{-6}$ (11·6–38)

Discussion

The present study shows that papaverine induced a potent dose-dependent vasodilatation in femoral arteries previously contracted with K^+. The maximal relaxation reached with this drug was greater than those obtained with the other compounds used. The responses caused by high concentrations of papaverine were increased by preincubation with MIX. Papaverine and MIX have a similar marked capacity to inhibit phosphodiesterase (Beavo et al 1971; Wells et al 1975; Kramer & Wells 1979), thus increasing the intracellular cAMP level to avoid its breakdown. In other vessels, the presence of a similar concentration of this xanthine (Gagnon et al 1980b; Salaices et al 1985) or 3·3 mM theophylline (Kukovetz et al 1982) either did not modify the papaverine-elicited relaxation or it was reduced. These authors suggest that due to the tissue phosphodiesterase being markedly inhibited by previous administration of xanthines, papaverine cannot act fully. In addition, it has been described that high concentrations of papaverine (from 10^{-5} M) may interfere with Ca^{2+} influx (Schümann et al 1975; Fujioka 1984; Huddart et al 1984). This effect could be in femoral arteries because from 10^{-5} M the dose–response curve changes slope sufficiently (Fig. 1). The increase of the response at high papaverine concentrations in the presence of MIX might be due to the additive effect of MIX on phosphodiesterase and papaverine on Ca^{2+} entry. $5{\cdot}5 \times 10^{-5}$ M MIX and the other concentrations used to determine the dose–response curve to this xanthine, seem to act essentially as an enzyme inhibitor, as has been obtained in other vessels (Kramer & Wells 1979; Gagnon et al 1980b). The fact that: (1) theophylline depressed Ca^{2+} uptake at very high concentration (4 mM) (Huddart et al 1983), and (2) the contractions caused by 75 mM K^+, being largely dependent on extracellular Ca^{2+} (Bolton 1979) in these arteries (Marín et al 1982), were slightly reduced or unaffected by this concentration of MIX, is in agreement with this.

Adenosine- and cAMP-elicited dose-dependent vasodilator responses in segments of femoral arteries, in agreement with the results obtained in other tissues (Napoli et al 1980; Gagnon et al 1980a,b; Toda et al 1982). The responses elicited by both drugs were increased by preincubation with MIX, as obtained in bovine coronary arteries (Napoli et al 1980). These results indicate that purinoreceptors placed on cell surfaces, are blocked by MIX and other xanthines (Gagnon et al 1980a,b; Toda et al 1982; Salaices et al 1985) and seem not to be involved in these responses. Alternatively, MIX could act by a different mechanism to block these receptors, probably by phosphodiesterase inhibitions (McKenzie et al 1977). The relaxant effects of adenosine may be produced by an interference with Ca^{2+} entry into the cell (Herlihy et al 1976; Fenton et al 1982). As the nucleoside did not alter the contraction elicited by K^+, this indicates that this mechanism seems not to participate in this relaxation. Another mechanism is by an intracellular increase in cAMP level. This may be produced as a consequence of adenosine interaction with surface purinoreceptors and subsequent adenylate cyclase activation (Blume & Foster 1975; Kukovetz et al 1978; Edvinsson & Fredholm 1983; Collis & Brown 1983) or by activation of an intracellular 'P site' insensitive to xanthines (Collis & Brown 1983). A cAMP-dependent mechanism seems to be involved in the responses caused by adenosine, as reported Napoli et al (1980), because: (1) they were increased by the presence of MIX, and (2) preincubation with this nucleoside enhanced the vasodilatation elicited by this xanthine (Figs 2, 3).

cAMP seems to have a different mechanism of action to that of adenosine, since it produces greater relaxant responses than the nucleoside and has a lower IC10. It has been reported that cAMP: (1) may penetrate cell membranes (Andersson 1972), though to a less extent than its analogue dibutyryl-cAMP (Bowman & Hall 1970; Mailman et al 1977), and consequently it produces a minor depressor effect; (2) may be metabolized both extra- and intracellularly to adenosine and 5′-AMP. These compounds can increase cellular cAMP levels (Andersson 1972; Kukovetz et al 1978), thus contributing to the response (Mailman et al 1977; Toda et al 1982); and (3) high concentrations of this cyclic compound may inhibit phosphodiesterase (Wells et al 1975).

The resemblance of the dose–response curves to cAMP and dibutyryl-cAMP (results not shown) indicates similar mechanisms of action and membrane penetration, thus increasing intracellular cAMP content. The increased response induced by this cyclic compound in the presence of MIX agrees with this assumption and with the possibility of phosphodiesterase inhibition, particularly at high cAMP concentrations, producing additive inhibition of the enzyme by exogenous nucleotide and MIX. The last hypothesis has been also suggested by Napoli et al (1980). However, in other vessels the response of this nucleotide was less than that elicited by adenosine (Bowman & Hall 1970; Mailman et al 1977; Toda et al 1982). They suggested that this is due to a poor capacity for cell entry or is induced by the metabolites produced. The relaxant effects caused by cAMP were reduced by preincubation of the arteries with adenosine. Similar desensitization has been obtained in guinea-pig fundic strips (Okwuasaba et al 1977).

Interpretation of these findings is difficult but could be due to the fact that both compounds act at similar sites in the chain of events occurring between increase in the cAMP content, that they seem to produce, and the reduction of free cellular Ca^{2+}. This reduction appears to be caused by stimulation of intracellular processes that control this Ca^{2+} level (Andersson 1972; Gagnon et al 1980a,b). The same decrease could be produced by MIX and papaverine (only in part since Ca^{2+} uptake is also depressed) (Gagnon et al 1980b). In addition it is noteworthy that this nucleotide and agents that enhance tissue cAMP such as adenosine, could produce vasodilatation by protein kinase activation (Napoli et al 1980; Silver et al 1984) and an antagonism between both agents may exist at this level. Further studies will be necessary to clarify the mechanism of action of adenosine and cAMP.

This work was supported by grants from C.A.I.C.Y.T. and F.I.S.S. The authors thank Dr I. M. Outschoorn for modifications in the text.

REFERENCES

Andersson, R. G. G. (1972) Acta Physiol. Scand. suppl. 382: 1–59

Beavo, J. A., Rogers, N. L., Crofford, O. B., Baird, C. E., Hardman, J. G., Sutherland, E. W., Newman, E. V. (1971) Ann. N.Y. Acad. Sci. 185: 129–136

Blume, A. J., Foster, C. J. (1975) J. Biol. Chem. 250: 5003–5008

Bolton, T. B. (1979) Physiol. Rev. 59: 606–718

Bowman, W. C., Hall, M. T. (1970) Br. J. Pharmacol. 38: 399–415

Collis, M. G., Brown, C. M. (1983) Eur. J. Pharmacol. 96: 61–69

Edvinsson, L., Fredholm, B. B. (1983) Br. J. Pharmacol. 80: 631–637

Fenton, R. A., Brutting, S. P., Rubio, R., Berne, R. M. (1982) Am. J. Physiol. 242: H797–H804

Fleming, W. W., Westfall, D. M., De la Lande, I. S., Jellet, L. B. (1972) J. Pharmacol. Exp. Ther. 181: 339–345

Fujioka, M. (1984) Br. J. Pharmacol. 83: 113–124

Gagnon, G., Regoli, D., Rioux, F. (1980a) Ibid. 69: 389–396

Gagnon, G., Regoli, D., Rioux, F. (1980b) Ibid. 70: 219–227

Herlihy, J. T., Bockman, E. L., Berne, R. M., Rubio, R. (1976) Am. J. Physiol. 230: 1239–1243

Huddart, H., Bayton, E., Shanklin, J. (1983) J. Exp. Biol. 107: 73–93

Huddart, H., Langton, P. D., Saad, K. H. M. (1984) J. Physiol. 349: 183–194

Kramer, G. L., Wells, J. N. (1979) Mol. Pharmacol. 16: 813–822

Kukovetz, W. R., Pöch, G., Holzmann, S., Wurn, A., Riuner, I. (1978) Adv. Cycl. Nucl. Res. 9: 397–410

Kukovetz, W. R., Pöch, G., Holzmann, S. (1982) Naunyn-Schmiedeberg's. Arch. Pharmacol. suppl. 321: R9

Mailman, D., Pawlik, W., Shepherd, A. P., Tague, L. L., Jacobson, E. D. (1977) Am. J. Physiol. 232: H191–H196

Marín, J., González, J. C., Salaices, M., Sánchez, C. F., Rico, M. L. (1982) Gen. Pharmacol. 13: 117–123

McKenzie, S. G., Frew, R., Bär, H.-P. (1977) Eur. J. Pharmacol. 41: 193–203

Napoli, S. A., Gruetter, C. A., Ignarro, L. J., Kadowitz, P. (1980) J. Pharmacol. Exp. Ther. 212: 469–473

Nielsen, K. C., Owman, C. (1971) Brain Res. 27: 33–42

Okwuasaba, F. K., Hamilton, J. T., Cook, M. A. (1977) Eur. J. Pharmacol. 46: 181–198

Salaices, M., De Gregorio, A., Marín, J. (1985) Gen. Pharmacol. in press

Schümann, H. J., Görlitz, B. D., Wagner, J. (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. 289: 409–418

Silver, P. J., Walus, K., Disalvo, J. (1984) J. Pharmacol. Exp. Ther. 228: 342–347

Toda, N. (1974) Ibid. 191: 139–146

Toda, N., Okunishi, H., Taniyama, K., Miyazaki, M. (1982) Blood Vessels 19: 226–236

Triner, L., Nahas, G. B., Vulliemoz, Y., Overveg, N. I. A., Verosky, M., Habif, D. V., Ngai, S. H. (1971) Ann. N.Y. Acad. Sci. 185: 458–476

Wells, J. N., Wu, Y. J., Baird, C. E., Hardman, J. G. (1975) Mol. Pharmacol. 11: 775–783