which occurred between 10^{-5} and 10^{-4} M for both compounds. The gradual relaxation phase was antagonized by propranolol while the steep relaxation phase was not. It is concluded that medroxalol and labetalol have β_2-adrenoceptor agonist activity which relaxes uterine muscle, and an additional relaxant activity which is unrelated to β-adrenoceptor activation.

We thank Dr D. L. Weiner for statistical analysis and Mr J. E. O'Leary III for technical assistance.

REFERENCES

Alquist, R. P. (1962) Arch. Int. Pharmacodyn. 139: 38–41

Brittain, R. T., Levy, G. P. (1976) Br. J. Clin. Pharmacol. 3: 681–694 Suppl.

Carey, B., Whalley, E. T. (1979a) J. Pharm. Pharmacol. 31: 791–792

Carey, B., Whalley, E. T. (1979b) Br. J. Pharmacol. 79: 13–15

Carpenter, J. R. (1981) Ibid. 72: 532P–533P

Cheng, H. C., Reavis, O. K. Jr., Grisar, J. M., Claxton, G. P., Weiner, D. L., Woodward, J. K. (1980) Life Sci. 27: 2529–2534

Dage, R. C., Cheng, H. C., Woodward, J. K. (1981) J. Cardiovasc. Pharmacol. 3: 299–315

Digges, K. G., Boyle, F. C. (1979) Br. J. Pharmacol. 67: 466P

Lands, A. M., Arnold, A., McAuliff, J. P., Luduena, F. P., Brown, T. G. Jr. (1967) Nature (London) 214: 597–598

Spedding, M. (1981) Br. J. Pharmacol. 74: 847P

Steele, R. G. D., Torrie, S. H. (1960) Principles and Procedures of Statistics. McGraw-Hill Publishing Co., New York.

van Rossum, J. M. (1963) Arch Int. Pharmacodyn. 43: 299–300

Winer, B. J. (1971) Statistical Principles in Experimental Design, 2nd Ed., pp 518–539. McGraw-Hill Publishing Co., New York.

J. Pharm. Pharmacol. 1982, 34: 195–196
Communicated September 1, 1981

0022–3573/82/030195–02 $02.50/0

Aminophylline-induced contractions of rabbit ear artery in high-K^+ Ca^{2+}-free medium

STEFANO MANZINI*, CARLO ALBERTO MAGGI, ALBERTO MELI, *Pharmacology Department Research Laboratories "A. Menarini" Pharmaceuticals, 50131 Florence, Italy*

The phasic plus tonic components of the bimodal response of rabbit ear artery to noradrenaline (NA) has been attributed to release of Ca^{2+} from cellular stores and mobilization of extracellular Ca^{2+} respectively (Bevan et al 1973; Steinsland et al 1973). Since methylxanthines produce contractures of both skeletal (Bianchi 1961; Endo 1975; Bianchi & Friedman 1979) and cardiac (Chapman & Leoty 1976; Matsumura & Narita 1980) muscle through Ca^{2+} release from sarcoplasmic reticulum (for a review see Fabiato & Fabiato 1977) it appeared worthwhile to determine the effect of aminophylline on rabbit ear artery under experimental conditions suitable for studying cellular Ca^{2+} mobilization-dependent contractions.

Methods

Male albino rabbits, 2·5–3 kg were anaesthetized with urethane (1·5 g kg^{-1} i.p.) and heparinized (1000 U.I. i.v.). A 3 cm segment of central ear artery was dissected free from adhering tissues, cannulated at both ends with polyethylene tubing and transferred to a 7 ml organ bath (at 37 °C) with a volume maintained constant by means of an overflow. The arterial segment was perfused intraluminally by means of De Saga 131900 six-channels peristaltic pump at a rate of 5 ml min^{-1} while extraluminal perfusion at a rate of 8 ml min^{-1} was obtained by means of a Mariotte bottle. Both intraluminal and extraluminal perfusion fluid were gassed with 95% O_2 and 5% CO_2 and heated at 37 °C. Changes in intraluminal perfusion pressure, recorded by means of a pressure transducer, were taken as an indirect measure of arterial contraction over the resting tone (20·4 ± 0·8 mmHg; n = 19). The artery was perfused intra and extraluminally with Krebs solution (mM) (NaCl 119, $NaHCO_3$ 25, KCl 4·7, $MgSO_4$ 1·5, KH_2PO_4 1·2, $CaCl_2$ 2·5, glucose 11). After 1 h stabilization period the perfusion fluid was replaced with high-K^+ Ca^{2+} free-solution (NaCl 69, $NaHCO_3$ 25, KCl 54·7 $MgSO_4$ 1·5 KH_2PO_4 1·2 glucose 11 mM) which produced a rapid contraction followed by return to basal values. Five minutes later intraluminal perfusion fluid was substituted with high-K^+ CA^{2+}-free solution containing NA or aminophylline at the desired concentration which produced a contraction followed by a return to resting values. Preliminary experiments showed that a 25 min perfusion with Krebs solution provided comparable responses to subsequent challenge with NA or aminophylline. When testing the influence of aminophylline (10^{-2} M) on contractions produced by a supramaximal (5×10^{-6} M) dose of NA, the procedure was similar to that described above with the difference than 2 min before NA challenge the inner perfusion fluid was substituted with a high K^+-Ca^{2+}-free solution containing aminophylline.

Results

In high-K^+ Ca^{2+}-free medium both NA ($1 \times 10^{-8} - 5 \times 10^{-6}$ M) and aminophylline ($1 \times 10^{-3} - 5 \times 10^{-2}$ M) produced a dose-dependent transient contraction with maximal values of 68·7 ± 2·2 and 17·49 ± 0·5 mmHg respectively (Fig. 1). The ED50 values calculated according to Litchfield & Wilcoxon (1949) where $7{\cdot}97 \times 10^{-8}$ M ($3{\cdot}92 \times 10^{-8} - 1{\cdot}62 \times 10^{-7}$) and $3{\cdot}15 \times 10^{-3}$ M

* Correspondence.

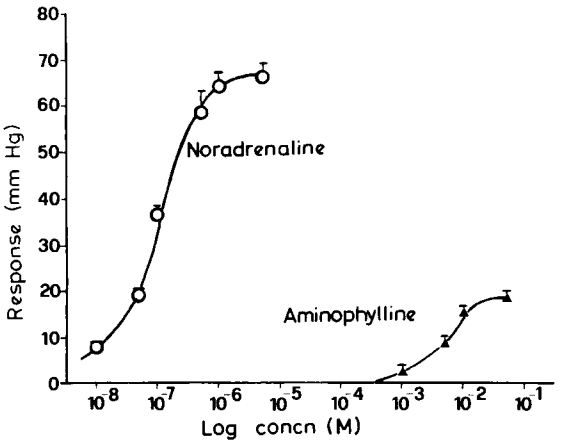

FIG. 1. Dose-response relationship for NA and aminophylline induced contraction in high-K^+ Ca^{2+}-free medium in rabbit ear artery. Each point represents mean ± s.e. of at least 6 experiments.

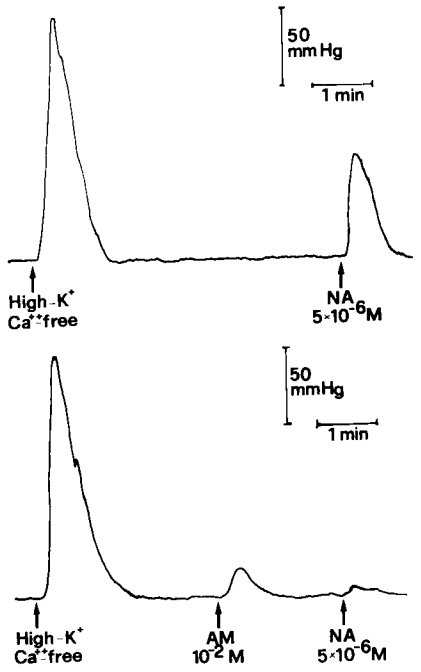

FIG. 2. Typical tracing showing the effect of aminophylline pretreatment on the contraction elicited by supramaximal dose of NA in high-K^+ Ca^{2+}-free medium.

($1{\cdot}03 \times 10^{-3} - 9{\cdot}64 \times 10^{-3}$) for NA and aminophylline respectively. Aminophylline pretreatment produced a 93·9 ± 1·8% (n = 6) inhibition of NA induced contractions (Fig. 2).

Discussion

It is well known that a high-K^+ medium induces mobilization of La^{3+}-sensitive, loosely bound, membrane Ca^{2+} stores (Weiss 1977), while Ca^{2+}-free medium eliminates trans-membrane Ca^{2+} movements. Therefore a contraction elicited in the high-K^+ Ca^{2+}-free medium should be strictly dependent upon the utilization of tightly bound cellular Ca^{2+} stores.

If this holds true, our results indicate that aminophylline is capable of mobilizing tightly bound cellular Ca^{2+} stores in smooth cells of the rabbit ear artery. Since Ca^{2+} release from sarcoplasmic reticulum is responsible for methylxanthine-induced contractures of both skeletal and cardiac muscles (Fabiato & Fabiato 1977) it is suggested that a similar mechanism might be responsible for aminophylline-induced contraction of the rabbit ear artery.

Although the possibility that aminophylline, at a concentration of 10^{-2} M, antagonized NA effects through phosphodiesterase inhibition cannot be ruled out, the hypothesis that aminophylline and NA utilize common Ca^{2+} stores in producing transient contraction in high-K^+ Ca^{2+}-free medium in rabbit ear artery is supported by recent findings (Deth & Lynch 1981) which indicate that in rabbit aorta caffeine and NA stimulate ^{45}Ca efflux through a similar mechanism.

REFERENCES

Bevan, J. A. Garstka, W. Su, C., Su, M. O. (1973) Eur. J. Pharmacol. 22: 47–53

Bianchi, C. P. (1961) J. Gen. Physiol. 44: 845–858

Bianchi, C. P., Friedman, H. A. (1979) Pharmacologist 21: 252

Chapman, R. A., Leoty, C. (1976) J. Physiol. (London) 256: 287–314

Deth, R. C., Lynch, C. J. (1981) Am. J. Physiol. 240 (Cell Physiol. 9): C239–247

Endo, M. (1975) Proc. Jpn. Acad. 51: 477–484

Fabiato, A., Fabiato, F. (1977) Circ. Res. 40: 119–129

Litchfield, J. T., Wilcoxon, F. (1949) J. Pharmacol. Exp. Ther. 96: 99–113

Matsumura, M., Narita, K. (1980) Jpn. J. Physiol. 30: 137–141

Steinsland, O. S. Furchgott, R. F., Kirpekar, J. M. (1973) Circ. Res. 32: 49–58

Weiss, G. B. (1977) in: Narahashi, T., Bianchi, C. P. (eds) Advances in General and Cellular Pharmacology, Plenum Press, New York, Vol. II pp 71–154