J. Pharm. Pharmacol. 1989, 41: 651–653
Communicated February 17, 1989

Putrescine reverses aconitine-induced arrhythmia in rats

CARLA BAZZANI, SUSANNA GENEDANI, SIMONETTA TAGLIAVINI, A. BERTOLINI, *Institute of Pharmacology, University of Modena, Via G. Campi 287, 41100 Modena, Italy*

Abstract—Putrescine, (150–300 mg kg^{-1} i.v.) injected into anaesthetized rats reversed aconitine-induced arrhythmia and restored sinus rhythm. In the same experimental model, quinidine and lignocaine had a transient therapeutic effect, procainamide was practically ineffective and verapamil worsened the aconitine arrhythmia, causing the death of all treated animals. These data demonstrate that putrescine has an antiarrhythmic effect in an experimental model particularly resistant to usual antiarrhythmic treatments.

Polyamines (putrescine, spermidine and spermine) are widely distributed polycations of key importance in the regulation of growth, development and function of various types of cells (Bachrach 1973; Heby et al 1975; Tabor & Tabor 1976; Jänne et al 1978; Pegg 1986). They are the only cellular cations whose concentration is controlled by specific biosynthetic and metabolic pathways (Cohen 1971; Pegg et al 1982; Pegg 1986). The rate-limiting enzyme in their biosynthesis is ornithine-decarboxylase (ODC) which catalyses the formation of putrescine from ornithine (Russell & Snyder 1968; Pegg & Williams-Ashman 1968; Moudsley 1979; Pegg 1986).

Besides their role in nucleic acid and protein synthesis (Schumkler et al 1975; Loftfield et al 1981), polyamines, as polycations at physiological pH, bind to cellular anionic sites, including cell membranes (Johnson & Bach 1968; Quigley & Cohen 1969), and stabilize membrane structure (Mager 1959; Herbst & Whitherspoon 1960; Anderson & Norris 1960; Harold 1964). This prompted us to study the effect of putrescine in an animal model of cardiac arrhythmia.

Materials and methods

Adult female Wistar rats (Morini, S. Polo d'Enza, Reggio nell'Emilia, Italy), (250–280 g) were anaesthetized with ethylurethane (1·25 g kg^{-1} i.p.), and the ECG recorded (lead II) (ETA-150 Cardioline, Elettronica Trentina, Trento, Italy). Arrhythmia was induced by the intravenous (i.v.) injection of aconitine (Sigma, St. Louis, MO, USA) at the dose of 50 μg kg^{-1}. Preliminary experiments had shown that, in our experimental conditions and with our strain of rats, such a dose of aconitine induces, a cardiac arrhythmia in about 50% of the treated animals, which starts within 1–2 min and continues unabated for over 60 min.

One min after the onset of the arrhythmia, animals received one of the following i.v. treatments: (a) 0·9% NaCl (saline); (b) putrescine [(butandiammonium-(1,4)dichloride), Merck-Schuchardt, München, FRG], 75, 150, 200 or 300 mg kg^{-1}; (c) quinidine (quinidine sulphate, Sigma, St. Louis, MO, USA), 1 or 5 mg kg^{-1}; (d) procainamide (Procamide Simes, Milano, Italy), 1 or 5 mg kg^{-1}; (e) verapamil (Isoptin, Knoll, Milano, Italy), 1 or 5 mg kg^{-1}; (f) lignocaine (lidocaine HCl, J. Monico, Mestre, Italy), 5 mg kg^{-1}. The effect of these doses of putrescine, quinidine, procainamide, verapamil and lignocaine, on the ECG of saline-pretreated rats was also studied.

At least 6 rats per group were used. Drugs (dissolved in saline), or saline, were injected in a volume of 0·5 mL kg^{-1}. ECG was recorded for 60 min after treatment, or until the death of the animal whichever occurred first. Data were analysed by ANOVA, Chi^2 test and ANOVA for tests of linear regression.

Correspondence to: C. Bazzani, Institute of Pharmacology, University of Modena, Via G. Campi 287, 41100 Modena, Italy.

Results

The arrhythmia induced by i.v. aconitine at the dose of 50 μg kg^{-1}, was continuous for over 60 min and was always compatible with survival, at least for the duration of the experiment (1 h). Aconitine-induced arrhythmia is characterized by early after-depolarizations, caused by increased residual i_{Na} during the plateau (Trautwein 1963; Peper & Trautwein 1967), and includes ventricular and supraventricular polytopic extrasystoles, bigeminy, and paroxysmal ventricular tachycardia. Putrescine, i.v. injected 1 min after the onset of the aconitine arrhythmia, had a clear dose-dependent anti-arrhythmic effect, with a linear regression ($P < 0{\cdot}01$) both between dose and time latency to arrhythmia resolution, and between dose and duration of arrhythmia resolution (Table 1). Some representative ECG recordings are shown in Fig. 1. Putrescine (150 mg kg^{-1}), restored sinus rhythm for a few seconds (11 ± 9) soon after injection, and then again and definitively 41 ± 5 min after injection; 200 mg kg^{-1} had a similar effect. At 300 mg kg^{-1} putrescine caused the almost immediate suppression of aconitine arrhythmia, which was replaced by marked sinus bradycardia and also by complete A-V block; normal sinus rhythm was definitively restored 28 ± 8 min after injection. Alone, putrescine induced some ECG changes (slowed A-V conduction, A-V block), lasting 1–2 min, only in 50% of the animals treated with the highest dose (300 mg kg^{-1}).

In our experimental conditions, only a high dose of quinidine, (5 mg kg^{-1} i.v.) induced a transient interruption of the aconitine-induced arrhythmia in 50% of treated animals; this antiarrhythmic effect started almost immediately after injection ($1{\cdot}00 \pm 0{\cdot}63$ min), and lasted 10 ± 8 min. One out of six treated animals died within the cut-off time of 60 min. Lignocaine (5 mg kg^{-1}) produced a resolution of the arrhythmia only in one of six treated rats, and for less than 1 min. Procainamide (1 or 5 mg kg^{-1} i.v.) was ineffective. Verapamil caused an abrupt worsening of the arrhythmia, with ventricular fibrillation followed by the death of all treated animals within about 1 min; at the doses used (1 and 5 mg kg^{-1} i.v.), verapamil alone caused only a transient slowing of A-V conduction and of repolarization, lasting a few minutes, without mortality.

Discussion

The present results show that putrescine, intravenously injected at doses of 75–300 mg kg^{-1}, dose-dependently reverses aconitine-induced arrhythmia in rats. Putrescine alone caused only very transient and minor ECG changes at the highest dose used. Aconitine-induced arrhythmia is particularly resistant to antiarrhythmic drugs (Trautwein 1963); indeed, in our experimental conditions, it was only temporarily reversed in half the treated animals by quinidine, while lignocaine was practically ineffective, procainamide was ineffective and verapamil increased aconitine cardiotoxicity and precipitated a ventricular fibrillation followed by the death of all treated animals.

The mechanism(s) of this antiarrhythmic effect of putrescine is(are) seemingly linked to its membrane tropism (Johnson & Bach 1968; Quigley & Cohen 1969) and membrane-stabilizing

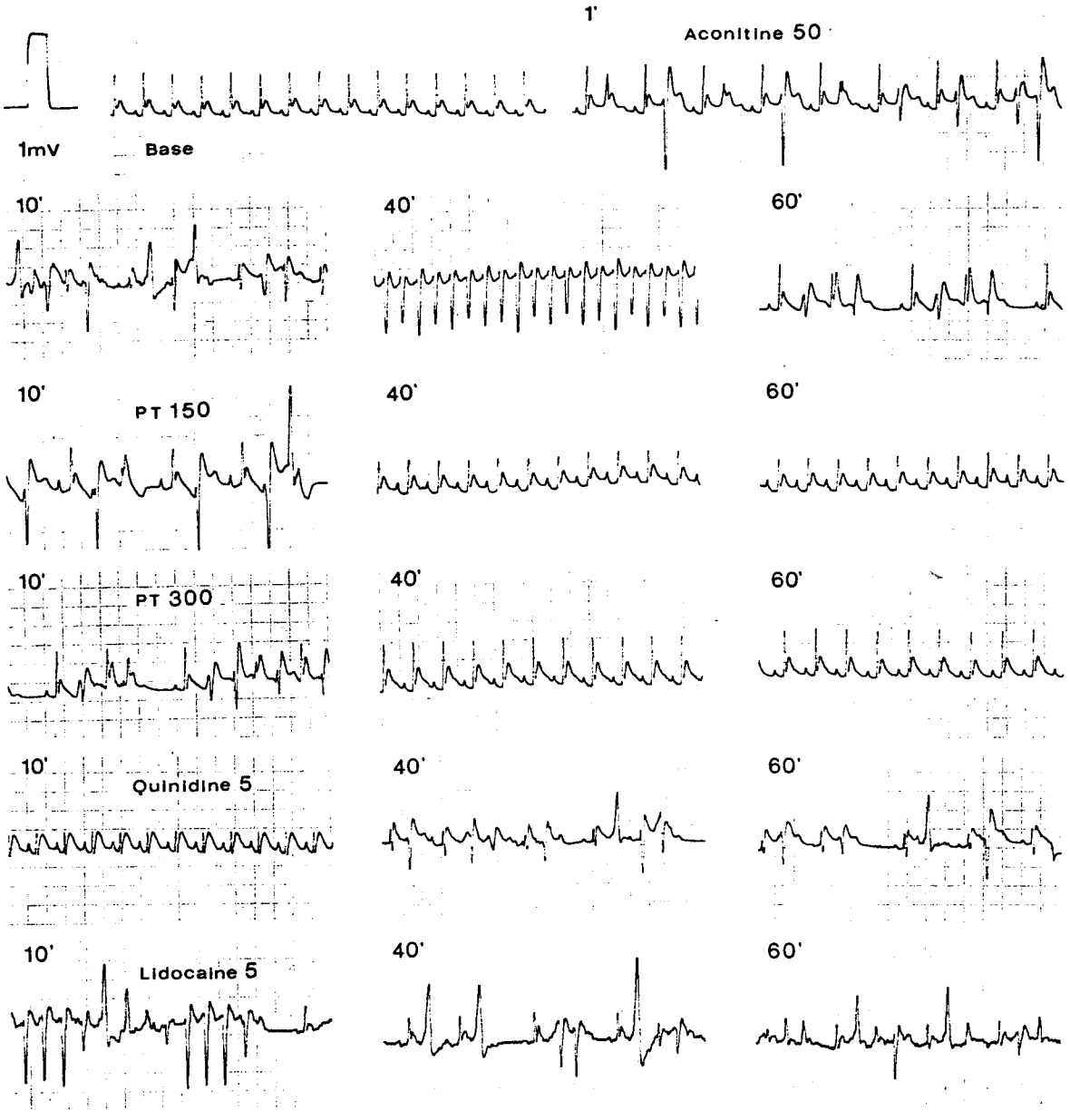

FIG. 1. ECG tracings from lead II. Influence of putrescine (PT) (150 or 300 mg kg^{-1}), quinidine (5 mg kg^{-1}) and lignocaine (5 mg kg^{-1}) on the arrhythmia induced by the i.v. injection of aconitine (50 $\mu g\ kg^{-1}$). Times from aconitine injection. Putrescine, quinidine and lignocaine were i.v. injected 1 min after the onset of the arrhythmia.

activity (Mager 1959; Herbst & Whitherspoon 1960; Anderson & Norris 1960; Harold 1964).

In the rat heart there are two distinct forms of ODC (Krelhaus et al 1975; Flamigni et al 1984), and the activity of this enzyme can be influenced by a large number of drugs, as well as by stressful conditions (adrenoceptor agonists, sympathetic stimulation, aortic and pulmonary artery constriction, forced immobilization, anoxia, etc.) (Flamigni et al 1986); accordingly, it has been suggested that polyamines may have a role in heart function (Flamigni et al 1986; Bazzani et al 1986). Cardiovascular effects of polyamines have been repeatedly described. In dogs, the i.v. injection of putrescine produces hypotension and tachycardia (Rossi et al 1984), seemingly due to histamine release and to reflex stimulation of carotid sinus baroreceptors; similar effects have been observed with spermidine and spermine (Marmo et al 1984). In the rat-heart-ventricle-strip preparation, putrescine has negative inotropic effect, whereas spermidine and spermine cause a sharp positive inotropic response followed by a rapid and more marked drop in the force of contraction (Bazzani et al 1986). The inhibition of heart polyamine synthesis reduces the positive inotropic effect of ouabain, noradrenaline and calcium (Bazzani et al 1988). In guinea-pig and cat isolated atria and in the guinea-pig papillary muscle, spermine accelerates the repolarization and spermidine increases the resting potential and the duration of action potential (Kecskemeti et al 1987). These authors suggest that polyamines influence cardiac membrane events: indeed, polyamines have been claimed to play physiological messenger role both in excitable (Shaw 1979) and in non-excitable cells, such as in the renal cortex (Koenig et al 1983).

The antiarrhythmic activity of putrescine described here is a pharmacological effect of this diamine which adds to other previously described analgesic (Genedani et al 1984) and antipyretic (Genedani et al 1986) effects.

Owing to the negligible acute toxicity of putrescine at the

Table 1. Aconitine-induced arrhythmia in rats. Influence of putrescine, quinidine, procainamide, verapamil and lignocaine. Aconitine was i.v. injected at the dose of 50 $\mu g\ kg^{-1}$. ECG was recorded for 60 min after treatment.

Treatment ($mg\ kg^{-1}$ i.v., 1 min after the onset of the arrhythmia)	% of animals with resolution of arrhythmia	Time of arrhythmia resolution (min after treatment; mean ± s.e.)	Duration of arrhythmia resolution (min; mean ± s.e.)	% survival
Saline, 1 $mL\ kg^{-1}$	0	>60	0	100
Putrescine, 75	33·3	50–52 (2/6)	3·33 ± 2·11	100
Putrescine, 150	100	40·83 ± 5·07*	19·33 ± 4·94*	100
Putrescine, 200	100	42·83 ± 4·88*	20·17 ± 4·99*	100
Putrescine, 300	100	28·00 ± 7·87**	32·00 ± 7·87*	100
Quinidine, 1	0	>60	0	80
Quinidine, 5	50	1·00 ± 0·63***	10·33 ± 7·70	83
Procainamide, 1	0	>60	0	100
Procainamide, 5	0	>60	0	100
Verapamil, 1	0	—	—	0
Verapamil, 5	0	—	—	0
Lignocaine, 5	16·67	15 (1/6)	5	100

*$P < 0.005$; **$P < 0.01$; ***$P < 0.001$ (ANOVA), compared with saline-treated rats.

doses used here in this antiarrhythmic effect, when confirmed and defined in other species, it might be considered for the treatment of human arrhythmias resistant to current antiarrhythmic drugs.

The skillful assistance of Dr Giovanni Pinelli is gratefully acknowledged. This work was supported in part by grants from Ministero della Pubblica Istruzione and Consiglio Nazionale delle Ricerche, Roma.

References

Anderson, N. G., Norris, C. B. (1960) The effects of amines on the structure of isolated nuclei. Exp. Cell. Res. 19: 605–618

Bachrach, U. (1973) in: Function of naturally occuring polyamines. Academic Press, New York pp 1–211

Bazzani, C., Genedani, S., Bertolini, A. (1986) Effect of polyamines on perfused rat heart contractility. Pharmacol. Res. Comm. 18: 503–512

Bazzani, C., Genedani, S., Tagliavini, S., Piccinini, G., Bertolini, A. (1988) Treatment with polyamine synthesis inhibitors reduces the positive inotropic effect of ouabain, noradrenaline and calcium. Ibid. 20: 23–35

Cohen, S. S. (1971) in: Introduction to polyamines. Prentice-Hall, Inc. Englewood Cliffs. New Jersey pp 1–179

Flamigni, F., Guarnieri, C., Caldarera, C. M. (1984) Characterization of highly purified rat heart ornithine decarboxylase. Biochem. Biophys. Acta 802: 245–252

Flamigni, F., Rossoni, C., Stefanelli, C., Caldarera, C. M. (1986) Polyamine metabolism and function in the heart. J. Mol. Cell. Cardiol. 18: 3–11

Genedani, S., Piccinini, G., Bertolini, A. (1984) Putrescine has analgesic activity, in rats. Life Sci. 34: 2407–2412

Genedani, S., Bernardi, M., Tagliavini, S., Bertolini, A. (1986) Putrescine has hypothermic and antipyretic activity, in rats. Ibid. 38: 1293–1298

Harold, F. M. (1964) Stabilization of streptococcus faecalis protoplasts by spermine. J. Bacteriol. 88: 1416–1420

Heby, O., Marton, L. J., Wilson, C. B., Martinez, H. M. (1975) Polyamines: a high correlation with cell replication. Febs Lett. 50: 1–4

Herbst, E. J., Whitherspoon, B. H. (1960) Inhibition of spontaneous and induced swelling of mitochondria by spermine. Fed. Proc. 19: 138

Jänne, J., Pösö, H., Raine, A. (1978) Polyamines in rapid growth and cancer. Biochem. Biophys. Acta 473: 241–293

Johnson, H. G., Bach, M. K. (1968) Uptake and subcellular localization of tritiated spermine in Escherichia coli. Arch. Biochem. Biophys. 128: 113–123

Kecskemeti, V., Kelemen, K., Marko, R., Selmeci, L. (1987) Modification of the cardiac transmembrane potentials and currents by polyamines. Eur. J. Pharmacol. 142: 297–303

Koenig, H., Goldstone, A., Lu, C. Y. (1983) Adrenergic stimulation of Ca^{2+} fluxes, endocytosis, hexose transport, and amino acid transport in mouse kidney cortex is mediated by polyamine synthesis. Proc. Natl. Acad. Sci. USA 80: 7210–7214

Krelhaus, W., Gibson, K., Harris, P. (1975) The effects of hypertrophy, hypobaric conditions and diet on myocardial ornithine decarboxylase activity. J. Mol. Cell. Cardiol. 7: 63–69

Loftfield, R. B., Eigner, E. A., Pastuszyn, A. (1981) Polyamines and protein synthesis. In: Morris, D. R., Marton, L. J. (eds) Polyamines in biology and medicine. Dekker, M., Inc., New York, Basel, pp 207–222

Mager, J. (1959) The stabilizing effect of spermine and related polyamines and bacterial protoplasts. Biochim. Biophys. Acta 36: 529–531

Marmo, E., Berrino, L., Cazzola, M., Filippelli, A., Cafaggi, G., Persico, N., Spadaro, R., Nisticò, G. (1984) Cardiovascular and respiratory effects of spermidine and spermine: an experimental study. Biomed. Biochim. Acta 43: 509–515

Moudsley, D. V. (1979) Regulation of polyamine biosynthesis. Biochem. Pharmacol. 28: 153–161

Pegg, A. E. (1986) Recent advances in the biochemistry of polyamines in eukaryotes. Biochem. J. 234: 249–262

Pegg, A. E., Williams-Ashman, H. G. (1968) Biosynthesis of putrescine in the prostate gland of the rat. Ibid. 100: 533–539

Pegg, A. E., Seely, J. E., Pösö, H., Della Ragione, F., Zagon, I. S. (1982) Polyamine biosynthesis and interconversion in rodent tissues. Fed. Proc. 41: 3065–3072

Peper, K., Trautwein, W. (1967) The effect of aconitine on the membrane current in cardiac muscle. Pfluegers Arch. 296: 328–336

Quigley, J. W., Cohen, S. S. (1969) The isolation of envelopes of Escherichia coli spheroplasts in the zonal ultracentrifuge. J. Biol. Chem. 244: 2450–2458

Rossi, F., Nisticò, G., De Marco, G., Berrino, L., Matera, C., Bile, G., Marmo, E. (1984) Cardiovascular effects of putrescine in dogs after systemic, intra-arterial vertebral and intraventricular injection. Res. Comm. Chem. Pathol. Pharmacol. 46: 43–51

Russell, D. H., Snyder, S. H. (1968) Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tissues. Proc. Natl. Acad. Sci. USA 60: 1420–1427

Schumkler, N., Jewett, P. B., Levy, C. C. (1975) The effects of polyamines on a residue-specific human plasma ribonuclease. J. Biol. Chem. 250: 2206–2212

Shaw, G. C. (1979) The polyamines in the central nervous system. Biochem. Pharmacol. 28: 1–6

Tabor, C. W., Tabor H. (1976) 1,4-diaminobutane (putrescine), spermidine, and spermine. Ann. Rev. Biochem. 45: 285–306

Trautwein, W. (1963) Generation and conduction of impulses in the heart as affected by drugs. Pharmacol. Rev. 15: 277–332