Interaction of polyamines or their precursors with the calcium-controlled secretion of peroxidase by sugarbeet cells

Th. Gaspar, C. Kevers, M. Coumans, C. Penel and H. Greppin

Hormonologie fondamentale et appliquée, Institut de Botanique B22, Université de Liège-Sart Tilman, B-4000 Liège (Belgium), and Laboratoire de Physiologie végétale, Université de Genève, 3, place de l'Université, CH-1211 Genève 4 (Switzerland), 15 July 1983

Summary. Three polyamines tested (cadaverine, spermidine and spermine) and their 2 precursors (the amino acids arginine and ornithine) inhibit the Ca^{2+}-mediated secretion of peroxidases by sugarbeet cells in suspension culture at concentrations ranging from 10^{-15} to 10^{-5}M. In the absence of exogenous Ca^{2+}, spermine added at higher concentrations mimics the activatory effect of Ca^{2+}, the other polyamines being without effect.

Exogenous polyamines (PA) have now been shown to stimulate growth in a wide range of plants[2]. It has been proposed that PA are responsible for the growth stimulation initiated by auxins in tissues[3]. It is also well known that PA has an effect on proteins, nucleic acids, and membranes[4-6]. Plant cells in suspension cultures secrete peroxidases in the presence of external Ca^{2+} [7,8]. Since auxins modify this calcium-mediated secretion[9], it was interesting to assay PA in this system.

Material and methods. Normal (auxin-requiring) non-organogenic callus of sugarbeet (*Beta vulgaris* L. altissima) was used. Experimental conditions for obtaining and maintaining these tissues in stock solid cultures have been reported elsewhere[10]. For cell suspension culture, inocula of such callus (1 ml packed cells) were taken during the exponential phase of growth, transferred into 150-ml flasks containing 25 ml of the medium used for callus, without agar, and dissociated by gentle stirring. The regulators used in the solid medium, benzylaminopurine and 2,4-dichlorophenoxyacetic acid, were added to the liquid medium at a concentration of 10 mg/l. The flasks were continuously shaken under continuous illumination (Sylvania Gro-lux fluorescent lamps, 3000 lx) at 20 °C[8]. For each assay, cells from suspension cultures (about 200 mg fresh wt) were resuspended in 2 ml of buffer containing 50 mM MOPS adjusted to pH 6.2 with Tris and 20 mM KCl. Each aliquot was left for 15 min without stirring. The PA cadaverine, spermidine and spermine (Sigma) were added to cell suspensions at concentrations varying from 10^{-15} to 10^{-3}M, using small volumes of stock solutions in water. PA, 1 mM $CaCl_2$, or PA and $CaCl_2$ were added at the beginning of the assay. Experiments with the amino acids arginine and ornithine were performed in the same way. During the assays, the cell suspensions were gently stirred at fixed intervals with a glass rod. Peroxidase activity present in the medium was measured by adding 50-µl samples to 2.5 ml of 40 mM phosphate buffer (pH 6.1) containing 8 mM hydrogen peroxide. The increase in optical density at 470 nm was read after 10 min. PA, arginine and ornithine have no in vitro effect on the peroxidase activity.

Results and discussion. The effects of increasing concentrations of the 3 PA cadaverine, spermidine and spermine on the peroxidase secretion by auxin-requiring sugarbeet cells were assayed. In the absence of Ca^{2+} (fig. 1, top), spermine enhances the peroxidase release by cells at rather high concentrations. Spermidine and cadaverine have a weak effect. In the presence of 1 mM $CaCl_2$ (fig. 1, bottom), the 3 PA exhibit an effect which is dependent on the concentration used. It must be emphasized that they inhibit the Ca^{2+}-mediated secretion of peroxidases at very low concentrations. The maximum inhibition, which is about 50% of the control, is observed for the 3 PA at 10^{-9}M. Higher concentrations are less inhibitory and at 10^{-3}M PA are almost ineffective. A comparison of the effect of spermine at this concentration without and with 1 mM Ca^{2+} shows

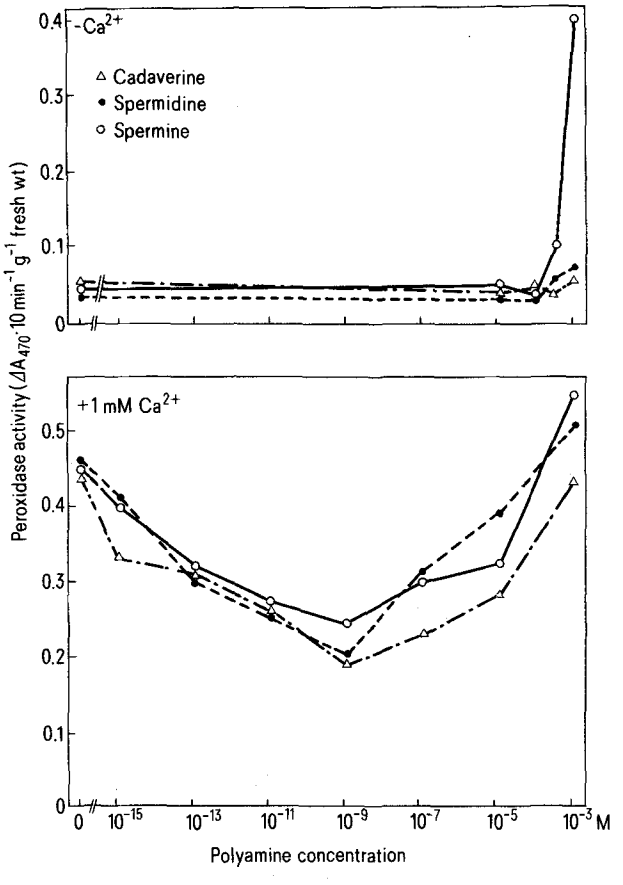

Figure 1. Effect of increasing concentrations of 3 polyamines on the secretion of peroxidases by sugarbeet cells in the absence (top) and presence (bottom) of $CaCl_2$. Peroxidase activity was measured in the media 90 min after the addition of PA and $CaCl_2$.

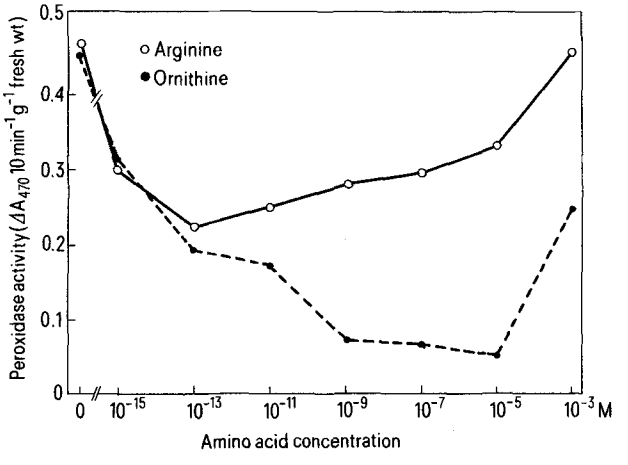

Figure 2. Effect of increasing concentrations of arginine and ornithine on the secretion of peroxidases by sugarbeet cells in presence of 1 mM $CaCl_2$. Measurements as in figure 1.

that spermine and Ca^{2+} effects are not additive. This suggests that spermine may act as a substitute for Ca^{2+} at high concentrations.

Two amino acids, arginine and ornithine, known to be the precursors of PA in plant cells[2], were also assayed. In the absence of Ca^{2+} they were without effect on peroxidase secretion. In the presence of Ca^{2+} (fig. 2), the general shape of the curve obtained with increasing concentrations of these 2 amino acids resembles that obtained with PA. The inhibition by arginine is about 50%. Ornithine, which is the most effective substance assayed in these experiments, reduces the effect of Ca^{2+} by more than 80%. It is not yet known whether the amino acids directly reduce the Ca^{2+}-mediated secretion of peroxidase or are previously transformed by cells into an active form. A kinetic study, which shows that both PA and amino acids are already active a few minutes after their addition (data not shown) suggests that the amino acids act directly on secretion without being metabolized.

It has already been shown with the same material that auxins have a short-term activating effect on the peroxidase secretion[9]. As a relationship between auxins and the level of PA in plant tissues has been reported[11], it may be envisaged that auxins control this secretory process by modifying the endogenous level of PA. The recent demonstration of arginine decarboxylase activity induction and PA accumulation by low pH[12] is in favor or this assessment. The mechanism of the effect of the interaction of PA with Ca^{2+} on enzyme secretion is not known, but it could be related to the functions of these compounds in the stabilization of membranes[2]. An electrostatic binding of the amine groups with the membrane phospholipids is likely, and this would be analogous to the electrostatic interaction found with the phosphate residues of nucleic acids[2].

1 Supported by the Belgian FRFC (grant No. 2.9009.75 to T.G.) and the Swiss National Foundation for Scientific Research (grant No. 3.140-0.81 to C.P. and H.G.).

2 Smith, T.A., News Bull. (Br. Pl. Growth Regul. Groups) *5* (1982) 1.
3 Bagni, N., Fracassini, S.D., and Torrigiani, P., in: Advances in polyamine research, vol. 3, p. 377. Ed. C.M. Caldarera. Raven Press, London 1981.
4 Atwar, V.J., Daniels, G.R., and Kuehn, G.D., Eur. J. Biochem. *90* (1978) 29.
5 Kaur-Sawhney, R., Altman, A., and Galston, A.W., Pl. Physiol. *62* (1978) 158.
6 Naik, B.I., and Srivastava, S.K., Phytochemistry *17* (1978) 1885.
7 Sticher, L., Penel, C., and Greppin, H., J. Cell Sci. *48* (1981) 345.
8 Kevers, C., Sticher, L., Penel, C., Greppin, H., and Gaspar, Th., Pl. Growth Regul. *1* (1982) 61.
9 Gaspar, Th., Kevers, C., Penel, C., and Greppin, H., Phytochemistry *22* (1983) 2657.
10 Kevers, C., Coumans, M., De Greef, W., Hofinger, M., and Gaspar, Th., Physiologia Pl. *51* (1981) 281.
11 Bagni, N., Malucelli, B., and Torrigiani, P., Physiologia Pl. *49* (1980) 341.
12 Young, N.D., and Galston, A.W., Pl. Physiol. *71* (1983) 767.

0014-4754/84/070696-02$1.50 + 0.20/0

Effect of platelet activating factor on guinea-pig papillary muscle

G. Camussi, G. Alloatti[1], G. Montrucchio, M. Meda[1] and G. Emanuelli

Laboratorio di Immunopatologia, Ospedale di S. Giovanni Battista e della Città di Torino, Torino (Italy), and Istituto di Fisiologia Generale, Università degli Studi di Torino, Torino (Italy), 2 August 1983

Summary. Platelet activating factor (PAF) induces a biphasic effect on guinea-pig papillary muscle: 1. a transient positive inotropic effect preceded by an increase in action potential duration (APD); 2. a marked negative effect on inotropism and on APD. Since Ca^{++} slow action potentials were initially enhanced by PAF and then markedly depressed, it is suggested that PAF specifically interferes with the Ca^{++} slow channel.

The platelet activating factor (PAF), a polar lipid mediator of inflammation was originally described as being released from basophils sensitized with Immune globulin E (IgE) after challenge with the specific antigen[2]. PAF has been recently defined as an acetyl glyceryl ether phosphorylcholine[3,4]. Subsequent in vitro studies have shown that besides basophils, polymorphonucleonuclear neutrophils (PMNs)[5], monocytes[5], macrophages[6], platelets[7] and endothelial cells[8] are capable of releasing PAF under immunologic and non-immunologic stimuli. An intravascular release of PAF has been documented not only during anaphylactic shock[9] but also in experimentally-induced immunocomplex pathology[10].

PAF, initially described as a potent platelet activator[2], is now known to possess a broad spectrum of biological activities, such as PMN aggregation, PMN and monocyte chemotaxis, stimulation of oxygen radical generation and lysosomal enzyme release (for review see Pinckard[11]). Recently an in vitro direct receptor-mediated spasmogenic effect on smooth muscle has been demonstrated[12].

The i.v. infusion of synthetic PAF not only induces a marked thrombocytopenia and neutropenia, but also reproduces the cardiovascular and respiratory alterations associated with the anaphylactoid reaction[13]. The mechanisms of PAF-induced cardiovascular alterations are as yet unknown. However, Burke et al.[14] recently demonstrated that PAF exerts a direct negative inotropic effect on isolated, perfused guinea-pig heart. The aim of the present report was to evaluate the effect of PAF on the electrical and mechanical activities of guinea-pig papillary muscle.

Materials and methods. Small papillary muscles isolated from the left ventricles of guinea-pigs (300–500 g, male) were placed in a small perspex chamber and perfused at 30 °C with gassed (95% O_2 and 5% CO_2) standard Tyrode solution (Na^+ 144.42 mM; K^+ 4 mM; Ca^{++} 2 mM; Mg^{++} 1.15 mM; Cl^- 141.25 mM; $H_2PO_4^-$ 0.42 mM; HCO_3^- 11.9 mM; D-glucose 5.6 mM; pH = 7.40). In order to study the Ca^{++} slow action potential, a K^+-enriched (K^+ = 22 mM) solution of identical composition was used.

Stimulation rate was 60 pulses/min in standard Tyrode solution and 10 pulses/min in K^+-enriched solution. Mechanical and electrical activities were evaluated respectively by an RCA transducer tube 5734 and a floating glass microelectrode as