Plant Physiol. (1992) 98, 1270–1276
0032-0889/92/98/1270/07/$01.00/0

Received for publication July 16, 1991
Accepted October 14, 1991

Ethrel (Ethylene Releaser)-Induced Increases in the Adenylate Pool and Transtonoplast ΔpH within *Hevea* Latex Cells

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ABSTRACT

The treatment of rubber tree (*Hevea brasiliensis*) bark with chloro-2-ethyl phosphonic acid (ethrel), an ethylene-releasing chemical, induced, after a lag period of 13 to 21 hours, a marked increase in the total adenine nucleotides (essentially ATP and ADP) of latex cells. This rise in the latex adenylate pool was concomitant with a marked decrease in the [ATP]/[ADP] ratio without significant changes in the adenylate energy charge. The apparent equilibrium constant for the adenylate kinase, which appeared to behave as a key enzyme in maintaining the adenylate energy charge in the latex, was considerably reduced, probably as a consequence of the alkalinization of the latex cytosol induced by the treatment with ethrel. To reduce the "sink effect" and activation of the metabolism induced in *Hevea* bark by regular tapping, the latex was collected by micropuncture (few drops) at increasing distance (5–50 centimeters) above and below an ethrel-treated area on the virgin bark of resting trees. The effect of ethrel was shown to spread progressively along the trunk. The increase in the adenylate pool (essentially ATP) was detectable as early as 24 hours after the bark treatment and was maximum after 6 or 8 days, 5 centimeters as well as 50 centimeters above and below the stimulated bark ring. The correlative vacuolar acidification and cytosolic alkalinization, *i.e.* the increase in the transtonoplast ΔpH, induced in the latex cells by ethrel were shown to be concomitant with the rise in ATP content of the latex. This suggests that the tonoplast H^+-pumping ATPase, which catalyzes vacuolar acidification in the latex, is directly and essentially under the control of the availability of its substrate (*i.e.* ATP) in the latex. The results are discussed in relation to energy-dependent activation of metabolism, and increased rubber production, as induced by the stimulation of rubber trees with ethrel.

The latex of rubber tree (*Hevea brasiliensis*) is the cytoplasm of specialized cells called laticifers located in the *Hevea* phloem (13). Rubber particles (*cis*-poly-isoprene globules) and small vacuolar structures called lutoids (12) constitute the large portion of the latex, that is exuded upon bark tapping. The metabolic activity in the laticifers must be sufficient to regenerate and compensate for the loss of cytoplasm (30–300 mL) upon each tapping (generally twice a week).

Sucrose supply and metabolism (30) were shown to play a major role in these regeneration processes. Sucrose is converted into mevalonate through the intermediate of acetyl-CoA in a NAD(P)H-dependent manner (17, 19, 20). Mevalonate is converted into isoprene units that polymerize to give natural rubber with consumption of ATP (20). ATP and ADP have been shown to be physiological regulators (2) in the whole metabolic pathway leading to biosynthesis of natural rubber (10, 17, 19, 20). Also, ATP is the specific substrate for a proton-pumping ATPase located on the latex cell tonoplast (lutoidic tonoplast) (9, 15). This H^+-ATPase is implicated in the regulation of pH of the latex cytosol (5, 22) and energizes the transport and accumulation of various solutes inside the lutoids (23).

The treatment of bark of the rubber tree with ethrel,[2] an ethylene releaser, efficiently "stimulates" the production of latex (rubber) (11). Stimulation of rubber production with ethrel has been shown to be associated with marked changes in the physiology and metabolism of the latex cells (6). In particular, treatment of the bark with ethrel induces an acceleration of sugar loading (21) and its catabolism (30) in addition to general increase in the protein turnover within the latex cells (6, 7). Stimulation with ethrel also induces, in the latex, a marked cytosolic alkalinization (7) coupled with an acidification of the vacuolar compartment (5, 6), which in turn is partly attributable to an activation of the lutoidic tonoplast H^+-ATPase (16). In fact, it seems that it is this cytosolic alkalinization that results in enhanced catabolism of sugars and latex regeneration in the laticifers by activating several pH-dependent enzymes (17, 18, 28).

The multifacet effect of ethrel on the metabolism of the latex cells trigged us to investigate the possible role of energetics as an important physiological control switch in the regeneration of latex. The present paper depicts the effects of ethrel treatments of *Hevea* bark on the adenine nucleotide pools and energetic parameters, in relation to the pH changes

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[2] Abbreviations: ethrel, 2-chloroethyl phosphonic acid; LDRC, latex dry rubber content; SDRC, sample dry rubber content; AEC, adenylate energy charge; AK, adenylate kinase; K_{app}, apparent equilibrium constant for an enzymic reaction.

of the cytosolic and vacuolar compartments in the latex, and the increase in the production of rubber.

MATERIALS AND METHODS

Plant Material, Ethrel Treatments, and Latex Collection

The latex was from 10-year-old rubber trees [*Hevea brasiliensis* (Kunth) Müll. Arg., GT-1 clone], regularly tapped in half spiral twice a week (1/2S d/3 6d/7), kindly provided by the IRCA Experimental Plantation, Bimbresso-Languededou, Ivory Coast (12 km from the laboratory).

First Experiment

For this experiment, in an attempt to follow the kinetic actions of ethylene treatments on the latex adenine nucleotide pools, rubber trees were selected for their homogeneous growth (circumference), rubber production, and biochemical properties of their latex. After some ultimate preliminary controls (cytosolic and vacuolar pH, sugar and total adenine nucleotide contents of their latex), they were left untapped for 10 d to recover. This allows the metabolism of their latex to reach a steady-state *in situ,* unaffected by the regeneration process that follows regular tapping, generally twice a week. Seven batches of three homogeneous trees were selected and treated with ethrel (5% in palm oil, 2 g/tree), on a 2 cm large scraped zone below their tapping cut, 71, 48, 33, 21, 13, 6, and 2 h before the "poststimulation tapping." The batches of three control trees were treated with palm oil alone (no ethrel).

All treated and control trees were tapped at the same time on the same day, to avoid any possible artifact due to daily variability. The first 10 mL fraction of the latex flowing immediately after the trees were tapped was discarded. Subsequently, independently for each tree, the following 25 drops of latex were collected in a glass tube containing 2.5 mL of a 0.1 N TCA buffer (300 mM mannitol, 25 mM Hepes-Tris, 0.1 mM ammonium heptamolybdate) in an ice bath, under continuous manual shaking. These conditions allow rapid dilution of the latex with only mild floculation of rubber, together with the immediate stop of its metabolism. As soon as the 25 drops had been collected, the floculated rubber phase of the diluted latex was coagulated in the field by vigorous shaking after the addition of 1 mL cold TCA (0.5 N TCA final concentration). The samples were kept cool in ice until extraction and analysis in the laboratory.

Fifteen milliliters of the latex flowing immediately after microsampling for adenine nucleotides analysis were directly collected in a glass tube cooled in an ice bath for pH measurements and determination of the LDRC. The remaining latex fraction flowing after the collection of the samples for analysis was collected independently at the end of the flow for volume and total yield determinations.

This type of experiment was performed twice (the first in September; the second in October).

Second Experiment

Six 10-year-old rubber trees were selected according to the same criteria as in the first experiment. Their tapping cuts were on the lower part of the trunk (less than 1 m from the ground). The trees were left untapped for 2 weeks to recover and allow their latex metabolism to reach a stationary state. The latex was sampled by micropunctures with steel pins above and below a ring (called "zero level") localized on the virgin bark, 1.2 m above the upper limit of the regenerating bark (Fig. 1). Three of the trees were stimulated at the zero level with 2 g ethrel (5%) on the scraped ring (2 cm large). Three other trees were used as control as described before.

In experiment 2-a, the latex was collected 20 cm above and below the zero level, day 1 before, day zero, then 12 h and days 1, 4, and 6 after the bark treatments, and analyzed as a mixed sample for each day and each tree.

In experiment 2-b, the trees were equipped with heat-sterilized stainless steel microgutters just beneath the place of each puncture (gently scraped into the surface). The latex was collected by micropunctures on a vertical line at different levels (5, 10, 25, 50 cm) above and below the zero level, and analyzed independently at day 0, 1, 4, 6, and 8 after the bark treatments.

In all cases, the 5 first drops of the latex flowing after micropuncture were discarded, and the 15 following ones were collected and immediately fixed as described above. The latex flow was stopped by applying to the pin holes, for about 1 min, a piece of cotton wool moistened with acetic acid (0.1

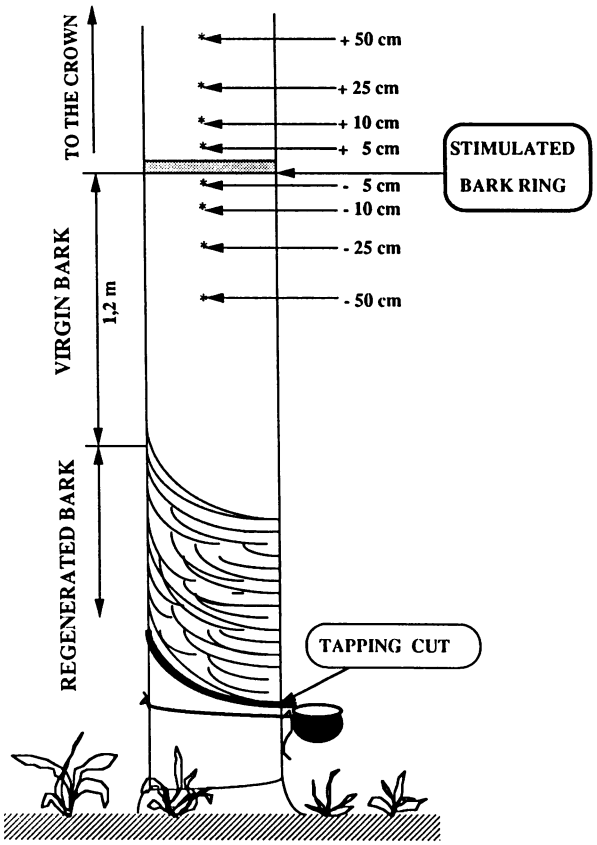

Figure 1. Schematic representation of a *Hevea* trunk, showing the location of the ethrel treatment as applied on a scraped ring of the virgin bark, and microtapping levels above (+) and below (−) the stimulated bark ring (second experiment).

N), which induces immediate coagulation of the rubber, to limit the extension of the drained areas.

This type of experiment was performed in November and December.

Determination of Adenine Nucleotide Pools

The latex of each tree was treated independently. The rubber coagulum obtained from the TCA (0.5 N)-fixed latex drops was stirred and squashed with a glass rod in order to release any adsorbed solute. Coagulated rubber was rinsed twice with TCA (0.5 N), then dried (24 h at 85°C) to determine the SDRC of each latex sample. The SDRC could serve as a reference to estimate, on the basis of the LDRC, the volume of the 15 or 25 latex drops collected for the determination of adenine nucleotides.

The TCA latex extracts were centrifuged (27,000*g* for 10 min at 4°C), and TCA was removed by three successive extractions with cold ether. The residual ether was evaporated by bubbling with nitrogen gas. The aqueous samples, which contain the equivalent of 15 or 25 drops of latex, were neutralized with (0.1 N) KOH, and the volume was adjusted to 10 mL with 30 mM Hepes-Tris buffer, pH 7.4.

ATP was quantified by a bioluminescence method using the luciferin-luciferase complex (25). An LKB-Wallack 1250 luminometer, equipped with an injection module and a digital display unit, was used. ADP and AMP were assayed by the same method after being phosphorylated to ATP using commercial pyruvate-kinase and adenylate-kinase, respectively. The concentrations of adenine nucleotides in the latex were determined in triplicate from standard curves using commercial pure nucleotides, after determination of inhibiting effects of the latex extracts on the bioluminescence efficiency. Adenine nucleotide concentrations are expressed in μM in the whole latex, based on the calculation according to the following formula:

$$\text{latex [AN]} = \text{ANs} \cdot \text{LDRC} \cdot \text{SDRCs}^{-1}$$

where latex [AN] is the concentration of a given adenine nucleotide in the whole latex expressed in μM in the latex; ANs is the total amount of a given adenine nucleotide in the 10 mL latex sample extract (15–25 latex drops), as expressed in μmol; LDRC is the latex dry rubber content (expressed in $g \cdot L^{-1}$); and SDRC is the sample dry rubber content, in the sample containing the 15 or 25 drops of latex (expressed in g).

The AEC is expressed according to Atkinson (2) as:

$$\text{AEC} = ([\text{ATP}] + 1/2[\text{ADP}]) \cdot ([\text{ATP}] + [\text{ADP}] + [\text{AMP}])^{-1}$$

Determination of the Cytosolic and Vacuolar pH

The cold latex samples were centrifuged at 4°C for 30 min (100,000*g*, Beckman Ti 50 rotor). The creamy supernatant consisting of the rubber particles was discarded, and about 3 mL of the clearest cytosol laying underneath the lighter rubber fraction were sucked up for direct pH measurements (Orion model 701 A digital pH meter equipped with an Ingold pH microelectrode). The bottom fractions essentially consisting of the vacuolar compartment (lutoids) were collected independently and sonicated (Heat Systems-Ultrasonic Inc., model W-225 R Sonicator equipped with a micro tip) at maximum intensity, for 15 s at 4°C, before pH measurements.

Rubber Yield Determinations

The rubber yields/tree were estimated from the total latex volume (including the samples for analysis) after each tapping and the LDRC measured after air drying 1 or 2 mL of the latex (24 h at 85°C).

Chemicals

The reagents were of the highest grade, either from Sigma or from Boehringer.

RESULTS

Kinetics of Early Effects of Ethrel, after Stimulation at the Tapping Cut and Latex Collection by Normal Tapping

Effects on the Energetic Parameters of the Latex

In this experiment, ethrel was applied at the tapping cut at different times while the stimulated as well as the control trees were tapped at the same time, on the same day, to correct for any tree-to-tree and daily variabilities.

The control treatment (Fig. 2A) with palm oil alone applied on the scraped bark exhibited very poor if significant effects (slight increase in the latex adenylate pool after 21 h) eventually attributable to wound. The results reported in Figure 2B do show that, compared with the control, stimulation with ethrel induced rapid and marked changes in the latex adenylate pools. The kinetics of ethylene effects could be subdivided into two phases.

An early stage, occurring as early as 2 h after stimulation with the ethylene releaser, was characterized by a marked decrease in the total adenylate pool in the latex. The ATP content in the latex from stimulated trees dropped by 30% below the control after 2 h, then 47% after 6 h. ADP was shown to stay more or less constant, whereas AMP exhibited an immediate increase as an early response to the treatment with ethrel. The total adenine nucleotide pool in the latex dropped by 22% lower than before the treatment, 6 to 13 h after the application of ethrel.

Following this transient decrease, a delayed phase was characterized by a marked increase in the adenine nucleotide pool in the latex. The ATP as well as ADP concentrations increased rapidly, exceeding the control level less than 21 h after treatment. Later, the ATP content in the latex from stimulated trees reached values higher than 200% (after 33 h) and then 240% (after 71 h) compared with the control. The ADP content increased by 400% over the control after 48 h.

Effects on the pH of the Two Major Compartments in the Latex

Figure 3 depicts the effects of stimulation with ethrel on changes of the cytosol and vacuolar (lutoidic) pH in the latex cell cytoplasm. The response to ethylene could be divided into two distinct phases as well.

An initial stage (less than 20 h duration) characterized by a

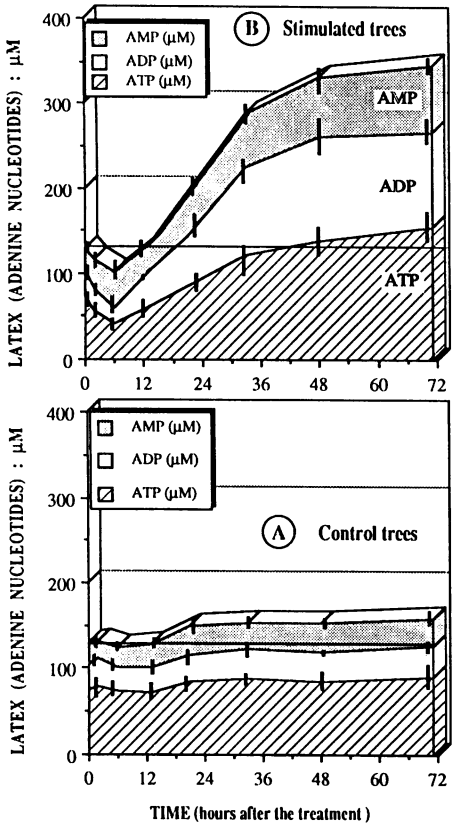

Figure 2. Kinetics of early effects of treatments with palm oil alone (A) and ethrel (B) when applied at the tapping cut of regularly tapped rubber trees, on the adenine nucleotide pool of the latex collected by conventional tapping (first experiment). Vertical bars represent the SE of six independent measurements (two independent experiments with three trees) performed in triplicate.

slight and slow alkalinization of the cytosol, and a slight transient rise (alkalinization) in the intravacuolar pH (maximum after 13 h). During this early stage, the pH response of both compartments varied in the same way. As a result, the transtonoplastic ΔpH was poorly affected or slightly decreased.

A delayed response characterized by a simultaneous alkalinization of the cytosol and acidification of the lutoids. Cytosolic pH increased by 0.42 unit compared with control (maximum after 33 h) and then remained 0.3 unit higher for more than 3 d. At the same time, the intravacuolar pH decreased by 0.2 to 0.3 unit compared with the control. The resulting transtonoplast ΔpH increased by 0.7 unit (33 h) and then 0.57 (71 h) unit compared with the control.

Effects of Ethrel Applied to the Virgin Bark on the Energy Parameters of the Latex Collected by Micropuncture

In the first experiment described above, the treatment with ethrel was applied at the level of the tapping cut, as for the classic methods used in *Hevea* stimulation, *i.e.* at the level of the regularly tapped bark.

Regular tapping of *Hevea* bark is known to induce a sink effect with an activation of the latex cell metabolism (29) that must constantly compensate for the loss of the exported latex. This activation of metabolism generated by tapping alone, at least in the area of the bark near the tapping cut, could interfere with the direct effects of the exogenous ethylene brought about by stimulation with ethrel.

The results obtained from Experiment 2-a and reported in Figure 4B depict the effects of ethrel applied to the virgin bark far from the tapped area (Fig. 1) on the adenylate pool in the latex collected by micropuncture.

The adenine nucleotide pool in the latex from virgin bark exhibited similar changes in response to ethylene, although the early response (12 h), corresponding to the decline in the adenylate pool (essentially ATP, whereas AMP was poorly affected), was much smaller. This initial stage, which one could consider here as a latency phase, was followed by a large increase in the ATP and ADP content in the latex that lasted more than 1 week. After 6 d, the adenylate pool in microsamples of latex from virgin bark treated with ethrel increased sixfold, which is even more than when the latex originated from regularly tapped trees. Treatment of the rubber trees with palm oil alone didn't bring about any significant change in the latex adenine nucleotide content (Fig. 4A).

From the results depicted in Figure 5, it is obvious that ethrel did affect the ATP/ADP ratio in the latex, which decreased by 50% of the control after less than 1 d and for more than 6 d, whereas the AEC remained unchanged. The K_{app} [(ATP)(AMP)/(ADP)^2] for the reaction catalyzed by adenylate kinase: 2 (ADP) → (ATP) + (AMP) calculated with the data obtained in this experiment, was decreased threefold after stimulation of bark. The control treatment (palm oil alone) did not bring about any significant change in the latex energetic parameters (not shown).

Further, we investigated (experiment 2-b) the distal effects of ethylene on the latex ATP when ethrel was applied on the virgin bark with minimized drained area (a few drops of latex were collected by micropunctures at opposite side of the

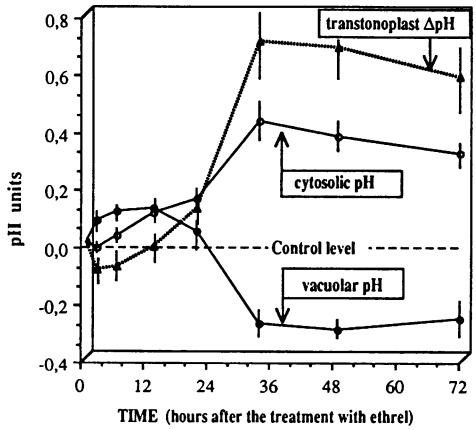

Figure 3. Kinetics of the ethrel effects on the cytosoiic and vacuolar pH, and on the resulting transtonoplast ΔpH within the *Hevea* latex. Ethrel was applied just below the tapping cut, and latex was collected by conventional tapping (first experiment). The results are expressed as Δ of the control (zero level). Vertical bars represent SE of six independent measurements (two independent experiments with three trees).

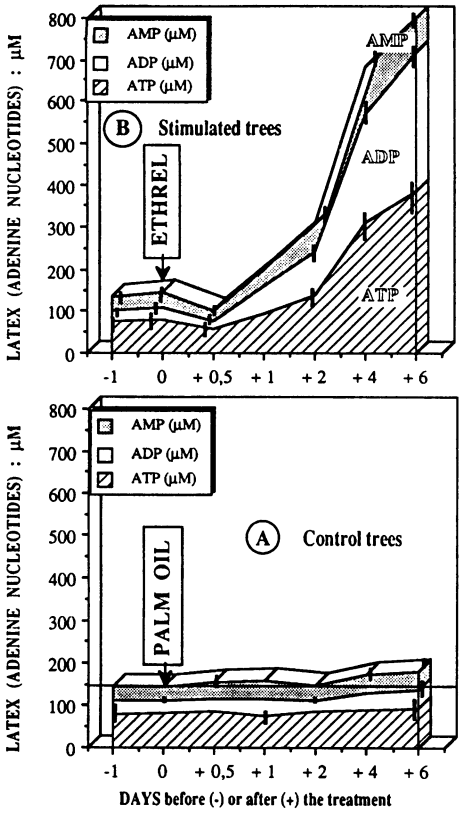

Figure 4. Kinetics of treatments with palm oil alone (A) and ethrel (B) when applied on a scraped ring on the virgin bark (see Fig. 1) on the adenine nucleotide pool in the latex collected by micropuncture, 20 cm above and below (mixed samples) the stimulated area (experiment 2-a). Vertical bars represent SE as in Figure 2.

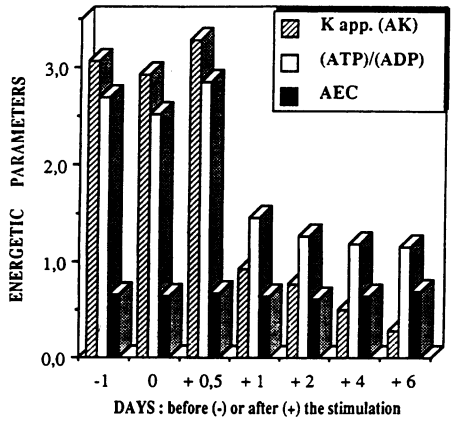

Figure 5. Kinetics of the ethrel effects, when applied on a scraped ring on the virgin bark (see Fig. 1), on the K_{app} for AK, on the [ATP]/[ADP] ratio and AEC in the latex collected by micropuncture 20 cm above and below (mixed samples) the stimulated area (experiment 2-a).

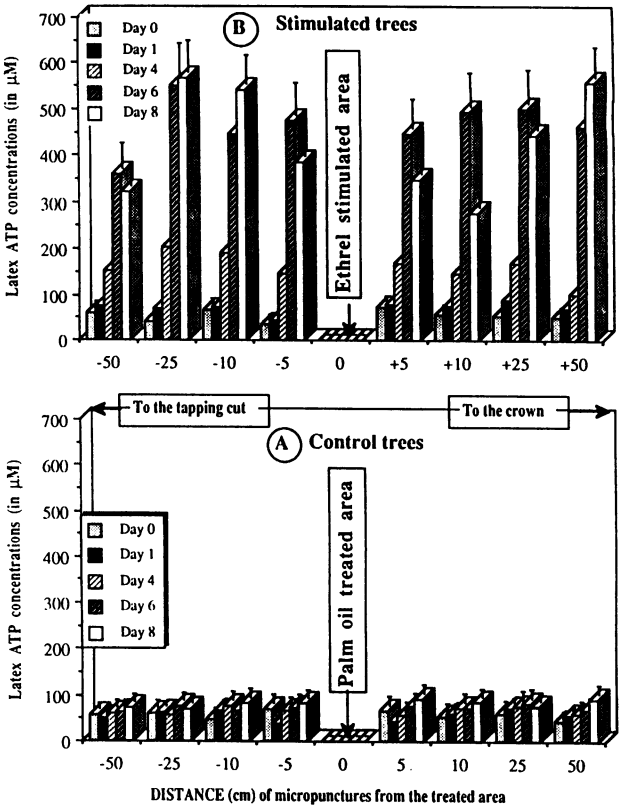

Figure 6. Kinetics of distant effects of treatments with palm oil alone (A) and ethrel (B) when applied on a scraped ring on the virgin bark (see Fig. 1), on the ATP content in the latex collected independently by micropuncture, 5 to 50 cm below or above the stimulated area (experiment 2-b). Vertical bars represent SE as in Figure 2.

trunk) (Fig. 1). The results reported in Figure 6B show that the increase in the latex ATP was detectable as soon as 24 h after the treatment, and spread immediately from 5 cm to more than 50 cm above and below the stimulated area. The ATP concentrations were maximal 6 or 8 d after the stimulation of the bark and reached values 8- to 10-fold higher than those of the controls (from 60–72 µM for the control or before the stimulation to 400–600 µM at day 6 or 8). Control treatments (palm oil alone) had no evident effects on the ATP content in the latex collected at the same distances above and below the treated area (Fig. 6A).

DISCUSSION AND CONCLUSIONS

Stimulation of rubber production with ethrel (the ethylene releaser) was shown to induce a reproducible large increase in the total adenine nucleotide content in the latex (essentially ADP and ATP) and a marked decrease in the ATP/ADP ratio without change in AEC. The slight increase (by 15%) in the adenylate pool in the latex from control trees might be ascribable to wound brought about by bark scraping, which is known to induce some slight release of endogenous ethylene in *Hevea* bark (6).

Yet, kinetic studies on the effects of stimulation with ethrel, as applied on the tapping cut of regularly tapped trees, showed

that its action on the latex adenylate pool is detectable after only a few hours following the stimulating treatment. These ethylene effects could be subdivided in two phases: (a) an initial step detectable as early as 2 or 6 h after the bark treatment and lasting about 13 h, corresponding to a transient more or less important decrease in the ATP and the total adenine nucleotide contents of the latex; and (b) a more delayed phase, which occurred between 13 and 21 h after the treatment and lasted more than 1 week, corresponding to a marked increase in the energy availability essentially through increase in ATP and ADP in the latex. These changes in the latex adenylate pool can be considered as the earliest events observed in response to a treatment of *Hevea* bark with ethrel (6, 7).

The increase in the latex adenylic pool was of higher intensity when ethrel was applied at the virgin bark far above the tapping cut. This is consistent with the assumption that this increase in energy availability in the latex is directly attributable to ethylene, independent of metabolic activation in the "sink area" induced by regular tapping (6, 29).

These stimulatory effects of ethrel applied to the virgin bark spread rapidly along the trunk, and was detectable more than 50 cm below and above the ethrel-treated area as soon as 24 h after the application of the stimulant. This effect of ethylene on the latex adenylate pool was shown to grow in intensity and to last more than 8 d after ethrel had been applied.

The total adenylate pool (essentially ATP) fell to nearly 22% as an early response to ethrel, especially when the latex originated from regularly tapped trees. This could be attributable to some true consumption of adenine nucleotide entities under an unmeasurable form by the method used here. This could be mobilization of ATP for an increased synthesis of RNAs that has been demonstrated in the latex in response to ethylene (6, 7) as in other stressed tissues (14).

An alternative could be that the net initial loss of adenine nucleotides reflects homeostasis of AEC via catabolism. Mammalian tissues and microorganisms stabilize AEC under stress by regulating adenine nucleotide pool size (4, 27). If *Hevea* latex cells react in a similar fashion, ethylene should increase ATP hydrolysis, producing ADP and AMP, followed by rapid dephosphorylation and/or deamination of AMP, thus reducing the total adenine nucleotide pool. AK would maintain a high proportion of ATP and sufficiently high AEC (3). For the same reason (AEC unchanged), the increase in the ATP and the total adenine nucleotide pools, as a delayed response to stimulation, could not be explained by ADP rephosphorylation alone. We suggest the occurrence of a *de novo* synthesis of adenine nucleotide entities induced as a delayed (after 21 h) response to ethylene. Reequilibration of AEC could then be mediated, here again, by AK, which is known to occur in the latex (1).

One striking effect of ethrel on these latex energetic parameters is the decrease in the K_{app} of the latex AK reaction, as calculated from the mass action ratio ([ATP][AMP] × $[ADP]^2$). It must be first pointed out that, as the K_{app} for AK in the latex from nonstimulated trees is about 3, this suggests that, contrary to most available data, AK in the latex does not function at the near equilibrium (3, 25). This equilibrium is displaced in favor of high ATP concentrations in the latex of nonstimulated trees. Stimulation with the ethylene releaser induced a marked decrease of the K_{app} for AK, from 3 to about 1, suggesting that equilibrium was approached. As AK is generally sensitive to pH and Mg^{2+} (24), this marked change in its K_{app} value under ethylene treatments might be attributable to the marked alkalinization of the cytosol, as confirmed here, and to lowered concentrations in free cytosolic Mg^{2+}, which has been reported in latex after stimulation with ethrel (6).

Stimulation with ethrel induces compartmental pH changes in the latex. These could not be essentially explained by the action of ethylene on the release of a variable amount of the vacuolar sap in the cytosol owing to lutoids bursting. Indeed, ethrel generally does not induce significant changes in the lutoids' stability in preexisting latex when stimulating the trees (*i.e.* the latex collected on the first tapping after treatment with ethrel as in the first experiment) (6). Furthermore, if very conflicting results have been reported concerning the lutoids' stability in the latex collected on the tappings following stimulation with ethrel (*i.e.* in the regenerated latex after the first tapping following the treatment), acidification of lutoids correlative with alkalinization of the cytosol under ethrel treatments has always been noticed (6). These simultaneous opposite changes in cytosol and vacuolar pH do suggest some *in situ* stimulation of tonoplast H^+-pumps by ethylene (5).

Recently, in addition to a highly active cytosolic alkaline PPase, a tonoplast PPase activity has been characterized in the latex (26) but, until now, its functioning as a H^+-pump could not be clearly established (JL Jacob, JC Prévôt, B Marin, unpublished). Yet, the functioning of the lutoidic tonoplast ATPase as a H^+-pump as implicated in generating and maintaining high transtonoplast $\Delta\mu H^+$ in artificial and physiological conditions has been clearly demonstrated (5, 8, 15, 22, 23) as well as its activation by ethylene (16).

The parallel increases in vacuolar acidification and cytosolic ATP content suggest that transtonoplast H^+-fluxes, as stimulated by *Hevea* bark treatments with ethrel, could essentially depend on the lutoidic ATPase activity (8, 22). This tonoplastic H^+-ATPase may be directly controlled by the availability of its substrate ATP in the latex. Indeed, the K_m of the lutoidic ATPase for MgATP was found to be about 500 to 700 μM when measured under physiological conditions (15), whereas the mean ATP content remained less than 80 μM in the total latex, that is to say less than 250 μM in the cytosol (about one-third of the latex volume) from nonstimulated trees. The latex tonoplast H^+-pumping ATPase thus seems to always operate at far less than its maximum potential *in vivo* because of poor ATP availability in the latex. As a consequence, its real activity should depend in a linear manner on cytosolic ATP concentrations. Then, the initial early decrease in latex ATP content (30–47% less than control) results in a decrease in ATPase real activity, despite some early stimulation of its specific activity through other processes (16), leading as a consequence to a transient vacuolar alkalinization. In contrast, the combination of the increase in the tonoplast ATPase specific activity (16), together with the marked increase in ATP concentrations in the latex cytosol, as later induced by ethylene, lead to effective activation of the tonoplast ATP-dependent H^+-pump, resulting in an increase in transtonoplast ΔpH.

Vacuolar acidification and high transtonoplast $\Delta\mu H^+$ favor

the detoxification of the cytosol, like Mg and citrate vacuolar accumulation and sequestration, for example (5, 23, 26). The conjunction of all these interdependent physiological events (increase in energy availability, alkalinization, and detoxification of the latex cytosol) induced by ethylene favor metabolism and, consequently, latex regeneration and production of rubber through increased sugar loading (21, 30), accelerated glycolysis (18, 28), and protein turnover (7).

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