Inhibition of cytokinesis in plant cells by 6-dimethylaminopurine and puromycin

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Summary. Binucleate cells are produced in garlic root tip cells with puromycin and with 6-dimethylaminopurine (6-DMAP), the purine component of puromycin. The possibility that the effect of puromycin on cytokinesis is due to 6-DMAP - which appears to be without short term effects on protein synthesis - is discussed.

Previous experiments have shown the effects of protein synthesis inhibitors on garlic root meristem cells[3-7]. All the drugs investigated induce a block of G_1-S and G_2-M transition during the cell cycle. Moreover cells in mitosis at the beginning of treatment progress in 1 of 2 ways: 1. Those in prophase cannot progress normally to metaphase; they are delayed for several hours then regress towards the interphasic state. 2. Those in metaphase, anaphase and telophase continue their progression to the interphasic G_1 state. In all cases, few binucleate cells are observed, indicating cytokinesis inhibition[8]. Among the various inhibitors of protein synthesis puromycin was examined for its effectiveness in the induction of binucleate cells. It may be supposed according to Appelman et al.[9] that the inhibition of cytokinesis is a side effect of the action of puromycin on a process other than protein synthesis. We therefore investigated the effects of puromycin and 6-DMAP on protein synthesis and binucleate cell formation.

Material and methods. Experiments were carried out on root meristems of garlic bulbs (*Allium sativum* L.). The roots were treated with puromycin (400 µg/ml) and 6-DMAP (300 µg/ml) for various periods. Mitotic indices (MI), percentages of prophases, metaphases, anaphases, telophases and binucleate cells were determined using fixed squashes. In labeling experiments, the roots were treated with puromycin (400 µg/ml) and 6-DMAP (300 µg/ml) for 30 min and 3.30 h and then incubated with both ^{3}H-leucine (2 µCi/ml for 30 min) (CEA France 10 Ci/mM) and the drugs. The incorporation of ^{3}H-leu in TCA precipitable material was determined by reference to the appropriate control samples (^{3}H-leu 30 min). Radioactivity was measured using an Intertechnique scintillation spectrophotometer SL 40.

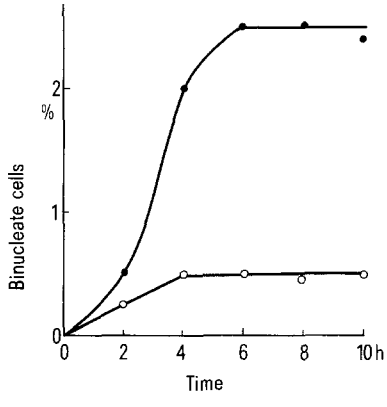

Fig. 1. Percentage of binucleate cells compared with the total cell population after treatment with puromycin (400 µg/ml) and with 6-DMAP (300 µg/ml). ○ puromycin, ● 6-DMAP.

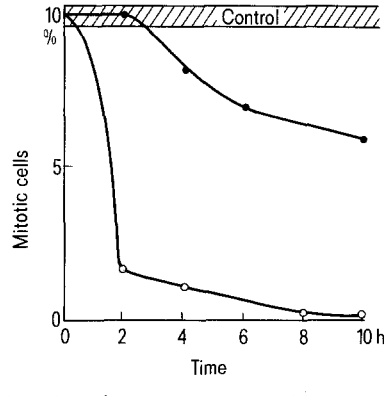

Fig. 2. Mitotic index (%) after treatment with puromycin (400 µg/ml) and 6-DMAP (300 µg/ml). ○ puromycin, ● 6-DMAP.

Results and discussion. In order to test the effect of puromycin and 6-DMAP on protein synthesis, the ^{3}H-leu labeled protein was assayed at various times after the beginning of the treatment period. The amount of ^{3}H-leu incorporated was reduced by puromycin after 1 h (33%). After 4 h of this treatment the incorporation clearly decreased (50%) while no such effect was noted in the presence of 6-DMAP.

The inhibitory effect on cytokinesis was determined by scoring the binucleate cells produced (figure 1) (The appearance of 2 nuclei in the same cytoplasm is the only definite evidence that cytokinesis has indeed been blocked). All the treatments used appear to inhibit cell plate formation within 2 h. However the percentage of binucleate cells in 6-DMAP experiments is significantly higher than that of puromycin treated cells. This difference reflects the fact that the mitotic index was immediately depressed and prophase to metaphase transition blocked by puromycin. This was not the case in 6-DMAP experiments. The results in figure 2 show that in puromycin experiments, mitotic indices which were about 10% in the control decreased considerably, to 1.8%, after a 2 h treatment. No significant delay in the entry of cells into mitosis was immediately observed in several experiments using 6-DMAP. The mitotic process appears to proceed normally for 4 h in the presence of 6-DMAP while G_2 cells are prevented from entering mitosis in puromycin experiments[8] In the table it can be seen that puromycin produces an accumulation and blockage of prophases. The percentage of prophases increases initially with time, while the percentages of metaphases, anaphases and telophases decrease during the whole process. In view of these results it may be concluded that prophases in puromycin treated

Frequencies per 100 mitotic cells of prophases, P, metaphases, M, anaphases, A, and telophases, T, after treatment with puromycin (400 µ/ml) and 6-DMAP (300 µg/ml) for 2, 4 and 6 h

h	IM	P	M	A	T
Control	10.0	50	11	12	27
Pu 2	1.8	60	10	9	21
Pu 4	1.1	77	0	1	22
Pu 6	0.7	100	0	0	0
6-DMAP 2	10.0	50	12	12	26
6-DMAP 4	8.0	57	9	6	28
6-DMAP 6	7.1	56	6	5	33

IM, mitotic index.

cells neither go through to metaphase nor remain blocked in prophase but regress to interphase. This effect is similar to that observed when treatment is carried out with other protein synthesis inhibitors under the same experimental conditions. In the presence of 6-DMAP no prophasic accumulation is observed, the telophase ratio, however, is markedly different between 6-DMAP and the control.

In this study 6-DMAP has been shown to have similar effects on cytokinesis but to differ from puromycin in that it does not affect protein synthesis and subsequently mitosis. Similarly, puromycin has been shown to inhibit cleavage in marine eggs[10] and Rebhun et al.[11] have suggested that puromycin, 6-DMAP and other substituted 6-purines affect the cleavage of marine eggs via the same process. To our knowledge neither puromycin nor 6-DMAP has previously been reported as blocking cell plate formation in plant cells. It was concluded that puromycin and 6-DMAP both interfere with cytokinesis in plant cells through a similar mechanism. In addition, the present results indicate that the physiological action of puromycin may involve the inhibition of protein synthesis and also other effects related to the purine moiety of puromycin.

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Male mouse submaxillary gland secretes highly toxic proteins

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Summary. Submaxillary gland saliva induced by phenylephrine from male mice was highly toxic to guinea-pigs, rats and hamsters, whereas the toxicity was relatively low to mice. One of the toxic components in the saliva was isolated as a kallikrein-like enzyme.

It is well known that there are marked sexual differences in the morphology and biochemistry of the submaxillary gland (SMG) of the mouse; the SMG of male is more abundant in serous-like granules in cells of the convoluted tubules[1,2] and in proteins such as esteroprotease[3], kallikrein[4], renin[5], nerve growth and epidermal growth factors[6,7] than that of female. Little is known, however, about the physiological significance of such sexual differences. SMG is generally considered to be an exocrine organ and recent studies have demonstrated that nerve growth and epidermal growth factors are also secreted into saliva[8,9]. We report here that SMG saliva elicited by phenylephrine from male mice is highly toxic to animals and that one of the toxic components in the saliva is a kallikrein-like enzyme.

Materials and methods. ICR-strain male mice, 14–16 weeks old, were anesthetized with pentobarbital and salivation was induced by injection of an α-adrenergic agent, phenylephrine (1 mg/kg, i.v.). The saliva elicited was collected by washing the oral cavity with 1 ml of distilled water using a pipet 10 min after phenylephrine injection. The protein content of the saliva was determined by the method of Lowry et al.[10]; an average of 5.2 mg protein can be collected per mouse in this way. The salivas collected from 1,600 mice were pooled, filtered through a 0.45 µm Millipore membrane and concentrated by lyophilization. SMGs were excised from 250 male mice aged 14 weeks. They were pooled, homogenized with 5 vol. of saline and the supernatant was obtained by centrifugation at 5000 × g for 30 min. The saliva and SMG extract were injected i.p. into test animals and LD_{50} was determined by the method of Litchfield and Wilcoxon[11] on the basis of the mortality rate within 24 h. Isoelectric focusing was carried out using a column with 110-ml capacity (pH range; 3 ~ 10)[12]. Kallikrein activity was measured by the 2 methods, a) hydrolysis of α-N-benzoyl-L-arginine ethyl ester (BAEE)[13], b) kinin-releasing activity from kininogen using rat isolated uterus[14]. Polyacrylamide gel electrophoresis was carried out in 7% gels at pH 9.5[15].

Results. Phenylephrine-induced saliva was highly toxic to guinea-pigs, rats and hamsters whereas the toxicity was relatively low to mice (table). The lethal effect appears within 24 h with weakness and prostration of test animals. Phenylephrine-induced saliva of SMG-ectomized male mice was nontoxic, indicating that the origin of toxic component(s) is

Toxic activity of phenylephrine-induced saliva and SMG extract of ICR-strain male mice

Species	Sex	LD_{50} (µg protein/g b.wt) Phenylephrine-induced saliva	SMG extract
Guinea-pigs	♂	6.6 (4.4–9.9)	24.1 (18.3–31.8)
Rats	♂	15.1 (10.4–21.7)	41.2 (29.9–56.9)
	♀	13.3 (9.8–18.1)	39.6 (30.7–51.1)
Hamsters	♂	26.3 (19.1–36.3)	80.9 (61.3–106.8)
Mice (ICR)	♂	127.6 (98.9–164.6)	380.0 (279.4–516.8)
	♀	119.4 (87.8–162.4)	321.6 (255.2–405.2)
Mice (C3H)	♂	95.3 (64.4–141.0)	286.2 (216.8–377.8)
	♀	84.7 (61.4–116.9)	241.8 (187.4–311.9)

Values in parenthesis shows 95% confidence limits. Hartley guinea-pigs, 4 weeks old, Wistar rats, 4 weeks old, Golden hamsters, 5 weeks old, and ICR and C3H mice, 4 weeks old, were used as test animals.