Dopamine Accumulation in *Papaver somniferum* Latex

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Organelles found in the 1000 × *g* fraction of latex taken from *Papaver somniferum* have been shown to accumulate dopamine to the exclusion of other catecholamines. Dopamine accumulation is both temperature and pH dependent and there is no evident requirement for ATP (1 mM) or Mg^{2+} (2 mM). The relatively high (4.37 mM), specificity of dopamine uptake, and insensitivity to inhibitors distinguish papaver latex organelle dopamine uptake from catecholamine uptake by mammalian chromaffin granules.

Introduction

Previous studies on the metabolic activity of *Papaver somniferum* latex, in particular the localization of enzymes and alkaloid metabolites, have shown that the alkaloid precursor, dopamine, occurs at significant levels [1]. Much of the dopamine is localized in the same dense vesicle fraction, those organelles which sediment at 1000 × *g*, as the alkaloids; however, the rate of uptake of [7-^{14}C]-dopamine into these fractions at room temperature is markedly different to that of morphine [1, 2].

Previous work on the occurrence of dopamine in plants is sparse and suitable models for the production and vacuolar storage of dopamine are not available. In animals, dopamine and other catecholamines are important in neurotransmission and are found stored in the adrenal medulla in neurotransmitter storage vesicles or chromaffin granules [3, 4]. The uptake of dopamine by these storage vesicles is highly dependent on temperature and has an absolute requirement for ATP and Mg^{2+}. Catecholamine uptake may be directly correlated to an inside-positive membrane potential (delta psi) and a proton gradient (delta pH) rather than ATPase activity [5–8]. Further it has been found that a specific monamine carrier mediates in the coupling between the generated electrochemical gradient and substrate accumulation [4].

Abbreviations: ATP, Adenosine-5′-triphosphate; BSA, Bovine serum albumen; CMB, 4-chloromercuribenzoate, DCCD, N,N′-dicyclohexylcarbodiimide; DIECA, diethyldithiocarbamate; FCCP, Carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazone; N–MM, N-methylmaleimide; EDTA, Ethylenediaminetetra-acetic acid.

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In the present experiments we have attempted to investigate the mechanism of accumulation of dopamine in *P. somniferum* latex 1000 × *g* organelles with a view to assessing its role as a plant metabolite.

Experimental

Plant material and isolation of 1000 × g organelles from latex

Papaver somniferum L. cult. var. Halle was grown in sequential batches in the departmental garden so that flowering occurred from June to September. Latex was collected and the 1000 × *g* organelles isolated as previously described [1]. In experiments where additions of ATP were made, the 1000 × *g* organelles were washed with buffer A at pH 7.0 (see below) twice to remove acid phosphatase and endogenous ATP [9] and were resuspended in buffer A at pH 7.0 so that 1 ml of the suspension was equivalent to 0.5 mg protein, determined according to Bradford [10], using BSA as a standard.

Chemicals

[7-^{14}C]-dopamine, 57 mCi/mmol, was obtained from Amersham International. All other chemicals were of high quality, obtained from various commercial suppliers.

Buffers

A, 200 mM phosphate 500 mM mannitol. pH as stated in the text.

B, 20 mM phosphate 0.1% Triton X-100 pH 6.2.

C, 200 mM Tris 500 mM mannitol. pH as stated in the text.

Assay procedure for dopamine uptake by 1000 × g organelles

In the standard experiment, dopamine equivalent to a final concentration of 1.75 mM ([7-^{14}C]-dopamine = 7×10^5 DPM) was added to 100–500 µl of $1000 \times g$ organelles in buffer A pH 7.0 and incubated at 25 °C for 60 min. The sample was then layered onto 20% sucrose in buffer A at pH 7.0 and centrifuged at $2000 \times g$ for 15 min to sediment the organelles. The resulting pellet was resuspended in 500 µl of buffer B for 30 min at 25 °C to plasmolyse the organelles. After centrifugation at $2000 \times g$ for 15 min to remove insoluble material, 100 µl of the resulting supernatant was counted, using 10 ml Aquasol scintillation cocktail and a Packard Model 3255 Tri-Carb Liquid Scintillation Spectrometer. This method reduced contamination by material not taken up by the organelles to less than 10% in zero time control samples and proved to be efficient way to handle short incubation times where necessary. When an inhibitor was used it was preincubated at 25 °C for 30 min with the organelle samples prior to addition of the labelled dopamine.

In each individual experiment, duplicate samples were used and the results given are averages of at least two experiments.

Assay of catecholamines by HPLC

In experiments to determine the specificity of dopamine uptake by the $1000 \times g$ organelles, uptake of non-radioactive catecholamines was monitored and quantified by HPLC [1].

Results and Discussion

In previous work [1] an attempt was made to separate the latex $1000 \times g$ organelles of *P. somniferum* into those which accumulate alkaloids and those which accumulate dopamine. To date, using continuous and discontinuous gradients, it has not been possible to show that separate storage organelles exist in *P. somniferum* latex although there is some evidence for different vacuolar storage of the compound in tissue cultures of *P. bracteatum* [11]. Discontinuous gradients were prepared by sequentially layering 200 mM phosphate buffer pH 7.0 containing sucrose 60% (7 ml) 50% (12 ml) 40% (12 ml) 30% (3 ml) in a 37 ml centrifuge tube. The level of dopamine which accumulates in the latex $1000 \times g$ organelles which sediment at the 50%–60% interface (Band B, [2]) on the discontinuous sucrose gradient is less by a factor of ten than the accumulated alkaloids in the same band. Levels of dopamine which remain in the supernatent from the $1000 \times g$ organelle preparation [1] are very much higher than the level of alkaloid; which may result either from the production of dopamine from dopa by dopadecarboxylase [12] in excess of uptake by the $1000 \times g$ organelles [2], or from the rupture of $1000 \times g$ organelles during handling. This last possibility, if correct, suggests that dopamine is accumulated in separate organelles from the alkaloids, since the ratio of alkaloid to dopamine in the supernatant is much lower than the same ratio in the $1000 \times g$ fraction.

Dopamine accumulation by the latex 1000 × g organelles accumulation with time

Dopamine is accumulated by a relatively slow process in that the accumulation of dopamine with time remains linear over the first 60 min at 25 °C (Fig. 1). This contrasts sharply with the rapid

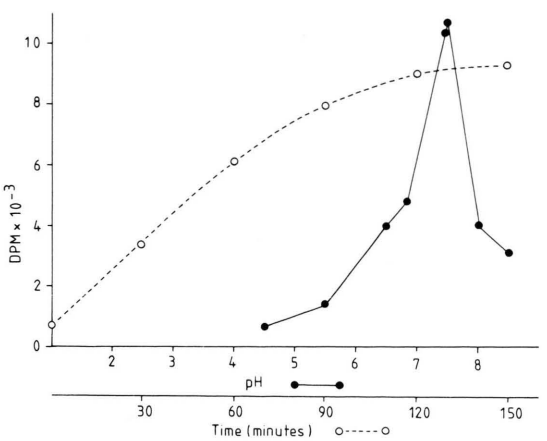

Fig. 1. Effect of pH and time on dopamine accumulation in *P. somniferum* latex $1000 \times g$ organelles. pH: 250 µl samples of $1000 \times g$ organelle suspension were centrifuged and the pellet resuspended in 500 µl of buffer A (pH 4.5–7.5) or buffer C (pH 6.85–8.5) and incubated for one hour at 24.5 °C with 0.5 µCi [7-^{14}C]-dopamine (57 mG/mmol). Time: 400 µl samples of $1000 \times g$ organelle suspension were incubated with 0.5 µCi [7-^{14}C]-dopamine (57 mCI/mmol) for the time indicated. – Counts per minute converted to disintegrations per minute (DPM) with standard efficiency curves, buffer C data was converted to buffer A base at pH 7.5.

accumulation of dopamine by the neurotransmitter storage vesicles in mammals, where accumulation is linear with time for only the first four minutes of exposure [3], as well as with the rapid accumulation of alkaloids by latex 1000 × *g* organelles [2].

The effect of temperature

Dopamine accumulation is sensitive to temperature and has maximal uptake into the organelles at 30 °C. In this respect, dopamine accumulation by latex 1000 × *g* organelles appeared to be similar to that of the mammalian storage vesicles [3], although in marked contrast to the accumulation of morphine by the latex 1000 × *g* organelles which is apparently temperature insensitive [2].

The effect of pH

Dopamine accumulation was found to be sharply affected by the pH of the external medium, exhibiting maximal activity at pH 7.5 (Fig. 1). The sharp fall off in activity at alkaline pH was in contrast to the situation with morphine accumulation where the latex 1000 × *g* organelles lost their ability to accumulate morphine, as well as to take up neutral red, below pH 4.5 but retained the ability to accumulate morphine to a pH of at least 8.5 [2]. Additions of 20 mM ascorbate and 50 mM DIECA to buffer C in a separate set of experiments at pH greater than 7.5 clearly demonstrates that the loss in activity is not attributable to oxidation of the dopamine at alkaline pH. The slight discoloration of the sample in experiments without antioxidant addition to the buffer suggested some organelle plasmolysis at alkaline pH; which, whilst it affected dopamine accumulation did not have an effect on the accumulation of morphine [2], further indication that morphine and dopamine storage may occur in different organelles within the 1000 × *g* organellar fraction.

Effect of ATP

The data obtained in measuring the effect of ATP and Mg^{2+} with EDTA as a Mg^{2+} chelator, is as shown in Table I. The addition of 1.0 mM ATP to a reaction mixture containing twice-washed organelles did not result in an increased dopamine uptake. 1 and 2 mM additions of Mg^{2+} with the ATP, as well as additions of EDTA with or without equimolar additions of Mg^{2+}, had no significant effect.

Uptake kinetics

The reported K_m for dopamine in rat brain storage vesicles is 0.26 μM, that for pig striatal vesicles is 6 times higher [3]. The apparent K_m for dopamine in *P. somniferum* latex 1000 × *g* organelles is 4.37 mM, as determined in this investigation. The improvement of the K_m through the use of ATP or Mg^{2+} was not attempted since no enhancement of dopamine uptake had been observed in the presence of ATP or Mg^{2+}.

Specificity of dopamine uptake

Mammalian chromaffin granules demonstrate an ability to take up dopamine, adrenaline, serotonin, or noradrenaline. Each of these compounds will compete inhibitively with any other, providing support for the concept that a single carrier is likely responsible for the transport of the several amines [13]. The uptake of alkaloids by 1000 × *g* organelles is limited to certain papaver alkaloids, with thebaine, codeine, and morphine being taken up with equal facility [2]. Uptake of catecholamines by the 1000 × *g*

Table I. Effect of ATP and Mg^{2+} and Mg^{2+} on dopamine accumulation by the 1000 × *g* organelles.

ATP [mM]	Additives [mM]		Dopamine Uptake % of control
1			94
1	EDTA	1	99
1	EDTA	2	89
1	Mg^{2+}	1	87
1	Mg^{2+}	2	91
1	EDTA	2	
	Mg^{2+}	2	86

1000 × *g* organelles were incubated with the additives for one hour at 25 °C, the ATP and radioactive dopamine sample were added and permitted to incubate for another hour.

Table II. Catecholamine uptake by 1000 × *g* organelles.

Catecholamine assayed	Uptake, % of increase in dopamine concentration
Adrenaline	17
Hordenine	0
Noradrenaline	8
Tyramine	22

Uptake is the ratio of an increase in the catecholamine content of the 1000 × *g* organelles to an increase in the concentration of dopamine, used as a control. Increase = final (after incubation) − initial (baseline) concentrations of organellar extract.

Table III. Inhibitor effects on dopamine uptake by $1000 \times g$ organelles.

Inhibitor	Inhibitor [μm]	Uptake, % of uninhibited control
None	–	100
Sodium vanadate	50	64
4-chloromercuribenzoate	50	121
	100	111
N,N′-dicyclohexyl-carbodiimide	50	113
	100	113
N-methyl maleimide	50	115
	100	108
Carbonyl cyanide 4-(trifluoromethoxy)-phenyl hydrazone	100	88
Ammonium chloride	1000	75

organelles demonstrated considerable specificity for dopamine as shown in Table II. The uptake of noradrenaline was less than 10% that of the weight of dopamine taken up by an organelle control sample in these experiments; in contrast, dopamine and noradrenaline are taken up to about the same extent by chromaffin granule membranes [14]. In the latex $1000 \times g$ organelles there was no evidence for the formation of noradrenaline from dopamine and therefore the existence of a membrane dopamine-betahydroxylase, as found in mammalian adrenal membranes [14], is unlikely.

Effects of inhibitors

The most effective inhibitor of morphine uptake by the $1000 \times g$ organelles was FCCP, which inhibited uptake by approximately 34% while vanadate only inhibited uptake by 18% [2]. Vanadate is primarily an inhibitor of mitochondrial ATPase, FCCP is an effective uncoupler and proton translocator. In the case of dopamine uptake, vanadate inhibits uptake by approximately 36%, while FCCP only inhibits by about 12%. While it has not been possible to distinguish between morphine and dopamine sequestering organelles it has been possible to distinguish between the characteristics of the two uptake systems. Thus, although the $1000 \times g$ organelles are essentially insensitive to CMB, DCCD, and N−MM inhibition insofar as both dopamine and morphine uptake are concerned, the uptake mechanism for the two classes of compounds, alkaloids and catecholamines, is distinctly different.

The accumulation of dopamine in the $1000 \times g$ organelle fraction of *P. somniferum* latex is of particular interest in view of its role as a prime precursor of the papaver alkaloids which, once formed, also accumulate in the $1000 \times g$ organelles. The presence of both dopamine and latex poly-phenolase [15] in the $1000 \times g$ organelles suggests that dopamine may also have a role in protection against penetration of the plant outer surface. During capsule damage, the latex exposed to the air forms a brown melanin-like substance which rapidly seals the wound. The release of both dopamine and poly-phenolase during injury, as a result of organelle plasmolysis at the air exposed surface, would suggest that dopamine has a prime role in this process as well as in the formation of alkaloids.

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