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IN VITRO CONVERSION OF MORPHINE TO ITS *N*-OXIDE IN *PAPAVER SOMNIFERUM* LATEX

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Abstract—Radio active morphine (^{14}C- and ^{3}H-labelled) was fed *in vitro* to freshly collected samples of capsules and stem latex of *Papaver somniferum* and it was shown that some of it was converted to radioactive *N*-oxide. Although metabolic activity and variation between samples of latex collected at different times were much less marked than those previously found using *in vivo* methods, the results do confirm that the isolated latex is a metabolically viable tissue.

INTRODUCTION

Previous work has shown that morphine is rapidly metabolized in the latex of the poppy plant (*Papaver somniferum*) particularly during the development of the seed capsule[1]. Some of the metabolites are more polar or have lower R_f values than morphine [2] and one of them has been shown to be nor-morphine [3]. Since morphine *N*-oxide has been shown to occur naturally in this plant [4] we have investigated whether this is also derived from morphine and therefore another product of morphine metabolism.

As the freshly collected latex is metabolically active *in vitro* [1] we used this tissue for all our experiments. These were also designed to determine whether *in vitro* metabolic activity varied with samples collected at different stages of plant growth and whether the stem latex was more active than that of the capsule [1].

RESULTS AND DISCUSSION

Samples of latex from capsules of known physiological age were collected at particular times and incubated with radioactive morphine in mannitol buffer. After incubation, the samples were extracted to exhaustion with methanol and aliquots chromatographed by prep-TLC. Bands corresponding to morphine and its *N*-oxide were separated, eluted and both the quantity of alkaloid present and its radioactivity determined.

The following results show clearly that some of the morphine had been converted into *N*-oxide: (a) In all 20 samples processed the TLC band corresponding to morphine *N*-oxide was distinctly radioactive with significantly less radioactivity on either side of the band. This rules out the possibility of the radioactivity being due to 'tailing' of the highly active morphine which has a higher R_f value than that of the *N*-oxide in the TLC system used. (b) There was a fairly good correlation between the sp. act. of the morphine and that of the *N*-oxide in any given sample; the higher that of morphine, the higher the *N*-oxide activity. (c) The *N*-oxide bands from the 12 × 1974 samples (Table 1) were eluted and combined. The average radioactivity, calculated

Table 1. Changes in morphine and morphine *N*-oxide radio- after incubation *in vitro* of [*N*-methyl ^{14}C] morphine in latex samples, collected simultaneously from stem and capsule at different stages of development

Age of capsule	Time of collection		Morphine* metabolized %	*N*-oxide† formed %
1974				
Wo +	10.00	stem	9	2.48
3 days		capsule	14	1.75
Wo +	10.00	stem	13	1.90
8 days		capsule	12	2.15
Wo +	08.00	stem	17	2.54
10 days		capsule	14	1.72
	10.00	stem	15	1.77
		capsule	10	1.87
	noon	stem	14	1.47
		capsule	10	1.39
	16.00	stem	20	1.51
		capsule	18	1.59
1975				
Wo +	08.00	stem	10	1.57
14 days		capsule	15	2.33
	10.00	stem	13	1.25
		capsule	8	0.90
	noon	stem	11	1.09
		capsule	21	2.88
	14.00	stem	18	2.28
		cpasule	10	1.83

Wo = Day on which petals were fully opened.
* Reduction in radioactivity of the morphine as % of total recovered radioactivity, per 100 mg latex.
† Radioactivity of the *N*-oxide as % morphine radioactivity in same sample.

from each band, was *ca* 39.5×10^3 dpm/mg. The elutes were evaporated to dryness and the solid matter converted into morphine by treatment with H_2SO_3; about 1 mg of the product was dissolved in 0.1 M HCl containing 50 mg cold morphine. The morphine was recrystallized 3 times, the two final recrystallizations giving values of 26.9 and 24.7 ($\times 10^3$) dpm per mg *N*-oxide after adjustment for the dilution factor and the loss of oxygen during reduction. (d) These results clearly indicated that the TLC band corresponding to morphine *N*-oxide contain this substance and that it was radioactive. However the experiments were performed with [*N*-methyl-^{14}C] morphine and in view of the possibility that removal of the radioactive methyl group and transfer to cold *N*-oxide may have occurred, similar experiments were done with morphine labelled in the nucleus ([1(*n*) − 3H] morphine). Furthermore morphine *N*-oxide tends to decompose to morphine and to more polar compounds during isolation and chromatographic manipulations and hence the following procedure was adopted for characterization and for ascertaining radioactivity. The band corresponding to morphine *N*-oxide was purified by repeated TLC and the MS of the resulting morphine product exhibited a weak $M^{+\cdot}$ ion at *m/e* 301 and a base peak at *m/e* 285 characteristic of morphine *N*-oxide [4]. An aliquot (3.4 mg) dissolved in MeOH was divided into two parts, one being reduced with H_2SO_3 as previously described [4] and the other used as a control. The reduced product and the control were banded on to silica gel plates and the resulting bands corresponding to morphine were eluted. The reduced product had an activity of 66×10^3 dpm and its MS was characteristic of morphine with a base peak at *m/e* 285 [4]. The corresponding band from the unreduced control had an activity of only 5×10^3 dpm.

Fluctuation of metabolic activity

The results in Table 1 do not show very marked variation in metabolic activity between the different sources of latex. Thus the amount of morphine metabolized ranged from 8 to 21% and the fluctuations in *N*-oxide formed varied from 0.9 to 2.54% of the radioactive morphine present. In view of experimental errors involved these *in vitro* variations are not highly significant and contrast with values of 80% or more for morphine metabolism [1, 2] and a marked change in morphine production before noon [5, 6] using *in vivo* methods. The physiological shock involved in separating the latex from the plant probably interferes significantly with normal metabolism.

Similar conversions of tertiary base to *N*-oxide have been reported in developing excised fruits of *Atropa belladonna*, when fluctuations varying between 12.5 to 30% conversion took place [7] and *Nicotiana* and *Senecio* species [8]. It is tempting to speculate that the formation of *N*-oxides is involved in *N*-demethylation and that in *P. somniferum* they are therefore involved in the formation of nor-morphine, the other known metabolite of morphine. Other possible functions of *N*-oxides in plants have been previously discussed [4, 7, 8].

EXPERIMENTAL

Spring sown plants of the Halle variety [1] of *P. somniferum* were used throughout. Samples of latex were collected by severing the capsule from the stem and immediately sucking up the exuding latex from stem and capsule with glass syringes (no needles). These samples were rapidly transferred to stoppered glass tubes containing mannitol buffer (mannitol 0.4 M, buffer pH 7, total molarity 0.5 M) containing either [*N*-methyl-^{14}C] morphine or [1(*n*)-3H] morphine. Each sample of morphine, purchased from the Radiochemical Centre, Amersham, U.K., was purified by band chromatography (system D, ref. [4]) and its radioactivity determined before use. The samples of morphine were of high sp act. and not more than 3 μg were added per 100 mg latex (containing about 5 mg endogenous morphine.) The latex + radioactive alkaloid was incubated in the dark at room temp. for 5 hr, excess MeOH was added and after vigorous shaking, filtered and made up to 25 ml. Suitable aliquots were run on TLC (systems C and D, ref. [4]) together with marker spots of morphine and morphine *N*-oxide. The corresponding bands were scraped off and their radioactivity determined by suspending in Cab-o-sil in phosphor [9]. The radioactivity of the remainder of the TLC plate was also determined so that a value for 'total recovered radioactivity' could be obtained. The quantities of alkaloid present were also determined when required [1]. For other purposes the bands of morphine or its *N*-oxide were scraped off and eluted with MeOH; where necessary the *N*-oxide was converted to morphine by reduction with H_2SO_3 [4].

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