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Effect of Ethylene and Carbon Dioxide on Potato Metabolism

STIMULATION OF TUBER AND MITOCHONDRIAL RESPIRATION, AND INDUCEMENT OF THE ALTERNATIVE PATH[1]

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ABSTRACT

The respiration of potato tubers (*Solanum tuberosum* var. Russet Burbank) which have been kept at room temperature for 10 days is stimulated upon subsequent treatment with C_2H_4 (10 microliters per liter) and O_2. The respiratory rise reaches a peak in 24 to 30 hours and thereafter declines. Coincident with the rise in tuber respiration is an increase in the respiratory rates of fresh slices and isolated mitochondria. Slices and mitochondria from C_2H_4- and O_2-treated tubers also display substantial resistance to CN, and the resistant respiration is inhibited by hydroxamates.

The longer the tubers are stored after harvest, the less effective is C_2H_4 in causing CN resistance in slices and mitochondria from treated tubers. Addition of 10% CO_2 to the C_2H_4-O_2 mixture, however, causes extensive CN resistance to develop, even in slices and mitochondria from old tubers. The results show that C_2H_4, O_2, and CO_2 act synergistically to induce alternative path development in potatoes.

The ability of C_2H_4 to stimulate the respiration of fruit and other plant tissues, including dormant potato tubers, is well documented (for a review, see 3). Since CN has similar effects on the respiration of potato tubers and many other plant organs (23–25), it has been suggested that C_2H_4 stimulation involves the engagement of the alternative, or CN-insensitive, respiratory chain of plant mitochondria (23, 26). Freshly cut slices from untreated tubers are largely sensitive to CN, but alternative path activity develops upon aging (7, 23, 26). The absence of CN resistance in fresh potato slices was thought to reflect the lability of the alternative path (23). However, it has been shown that C_2H_4 treatment of dormant tubers results in CN-resistant fresh slice and mitochondrial respiration, and it has been suggested that the alternative path is not initially present in the tuber, but develops upon C_2H_4 treatment (21, 22).

The present paper reports a more detailed investigation of C_2H_4-induced respiratory changes in potatoes. While the results of Rychter and colleagues (21, 22) are confirmed, we further find that CO_2 is synergistic with C_2H_4 (and O_2) in inducing the alternative path, and that an increase in the specific mitochondrial respiration rate attends the respiratory rise observed in the tuber.

MATERIALS AND METHODS

Untreated potato (*Solanum tuberosum* var. Russet Burbank) tubers were generously provided by H. Timm, University of California, Davis. Tubers were stored at 7 C. Unless otherwise indicated, for approximately 10 days prior to use tubers were held at room temperature in a chamber through which humidified air was blown. Subsequently, tubers were treated with gases as described (24) and CO_2 evolution was monitored with a Beckman CO_2 gas analyzer. O_2 consumption by tissue slices (4 g) was measured either with a Clark O_2 electrode in 25 ml of 10 mM phosphate buffer (pH 7.0), or manometrically (14). Mitochondria were prepared by published methods (12) and purified by centrifugation (25,000 rpm [82,000*g*] in a Beckman L5-50 ultracentrifuge, SW 27.1 rotor, for 3 hr) on linear sucrose gradients (20–60%). The mitochondria were located in a single band with an average density of 1.186. They were removed with a large bore syringe, carefully diluted to 0.4 M sucrose by dropwise addition (with continuous stirring) of 10 mM TES buffer (pH 7.2) + 0.1% BSA, and pelleted at 8,000*g* for 10 min. Final resuspension was in 25 mM TES buffer + 0.4 M mannitol and 0.1% BSA. Mitochondrial O_2 consumption was measured polarographically in a standard reaction medium of 0.4 M mannitol, 25 mM TES (pH 7.2), 5 mM $MgCl_2$, 5 mM KH_2PO_4, and 0.1% BSA (12). When succinate was substrate, 0.1 mM ATP was included in the reaction medium to activate succinic dehydrogenase. Protein was estimated by the method of Lowry *et al.* (16), and ADP/O ratios according to Chance and Williams (4).

RESULTS

Effect of C_2H_4 on Tuber Respiration. Tubers treated with 10 μl/l C_2H_4 in pure O_2 show a dramatic increase in CO_2 output after a lag of several hr (Fig. 1) (*cf.* 5, 20, 25). Treatment with O_2 alone has no effect (Fig. 1), while treatment with C_2H_4 in air causes somewhat less than half the respiratory rise in C_2H_4 and O_2. That is, C_2H_4 and O_2 are synergistic in their effects (5). Tuber respiration in the presence of C_2H_4 and O_2 reached a peak in about 30 hr after C_2H_4 application (some variation was observed), and declined thereafter to a value slightly above the control (O_2-treated) rate (Fig. 1). Removal of the tubers from C_2H_4 after the peak had little effect on the subsequent decline in respiration (Fig. 1). Reid and Pratt (20) and Huelin and Barker (8) made similar observations, and found that the tubers regained their responsiveness to C_2H_4 only after several days in air.

Effect of C_2H_4 on Slice and Mitochondrial Respiration. Fresh slices from C_2H_4- and O_2-treated tubers also show an increase in respiratory rates over a 30-hr period (Table I). Concurrently, slice respiration becomes increasingly resistant to CN (as well as to antimycin; data not shown). After 34 hr, O_2 consumption by slices from C_2H_4-treated tubers is 90% insensitive to 0.1 mM KCN (Table I). Whereas slices from control tubers also show some resistance to CN (Table I), the resistant component of slice respiration in this case is insensitive to hydroxamic acids and is termed "residual" respiration (13). The nature of this respiration is not known, but is constant in both treated and untreated slices (Table III),

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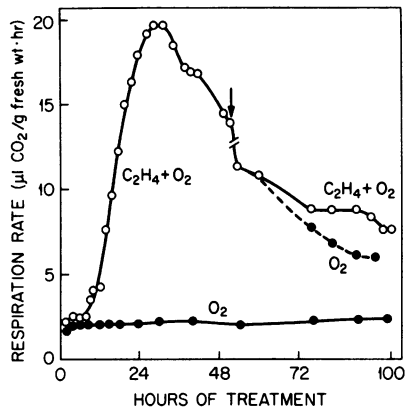

FIG. 1. Effect of C_2H_4 and O_2 on tuber respiration. Tubers were treated with O_2 or 10 µl/l of C_2H_4 plus O_2, as described under "Materials and Methods," as CO_2 evolution was continuously monitored. At the time indicated by the arrow, a number of the C_2H_4-treated tubers were transferred to O_2 alone. (○——○): tubers in C_2H_4 + O_2; (●——●): tubers in O_2.

climacteric-like rise in tuber respiration, which is accompanied by a similar rise in specific mitochondrial activity and a development of the alternative path.

Effect of CO_2 on Slice and Mitochondrial Respiration. The results discussed above were obtained relatively shortly (approximately 2 months) after tuber harvest. As the length of the storage period increased, we found it increasingly more difficult to repeat them. Not only did the rise in tuber respiration decline, but slices and mitochondria showed less resistance to CN (Table II).

Lange (11) and McCaig and Hill (19) have shown that CO_2 can lead to development of CN resistance in potato slices and wheat coleoptiles, respectively. We tested the effect of 10% CO_2 on the C_2H_4-induced CN insensitivity in slices from treated potato tubers. The presence of 10% CO_2 during O_2 treatment had no significant effect on slice respiration or mitochondrial resistance to CN (which remained nil; Table III). When CO_2 was added to the C_2H_4-O_2 mixture, however, slice respiration was depressed slightly but became almost completely insensitive to 0.1 mM CN, even in the presence of uncoupler (Table III). Uncoupler was used in these experiments to minimize the significance of the residual respiration, and to elicit maximum respiratory rates. In the absence of

Table I. Effect of tuber treatment with ethylene and oxygen on slice respiration

Tubers were treated as described in legend for Figure 1. At the times indicated, sample tubers were withdrawn and slices cut. Oxygen consumption was measured polarographically as described in Materials and Methods. KCN (0.1 mM) was added after a steady rate of oxygen uptake had been obtained. Resistance to cyanide is expressed as total resistance (i.e. without residual rates subtracted). Ethylene concentration was 10 µl/l where shown.

Length of tuber treatment	Tubers treated with O_2			Tubers treated with C_2H_4 plus O_2		
	Slice respiration		Resistance	Slice respiration		Resistance
	Control	+ KCN		Control	+ KCN	
hours	µl O_2/g fresh wt·hr		%	µl O_2/g fresh wt·hr		%
0	25	6	24	25	6	24
12	-	-	-	30	8	27
24	18	4	22	28	16	57
34	-	-	-	40	36	90
49	22	6	27	38	26	68
58	-	-	-	40	32	80
73	26	6	23	40	20	50
97	18	3	17	36	12	39

and in aged slices (13). Here, we are interested only in SHAM[2]-sensitive, CN-resistant respiration, and in Tables II, III, and IV, CN resistance is coextensive with SHAM sensitivity. Addition of 1 to 2 mM SHAM reduces the CN-resistant respiration of slices from C_2H_4-treated tubers to values close to the residual rates.

The CN resistance of slice respiration declines coincidentally with tuber respiration rates (Fig. 2; *cf.* Fig. 1). Mitochondria isolated from C_2H_4-treated tubers also develop and subsequently lose CN resistance, in parallel with the slices (Fig. 2). In all experiments, this close association was observed between the CN resistance of slice and mitochondrial respiration (Table III). Previous results have shown that C_2H_4 induces alternative path development in potatoes (21, 22). C_2H_4 also causes a considerable increase in specific mitochondrial respiration (Fig. 3; see also Table VI), and the latter shows the same pattern of development as does tuber respiration (Fig. 3, *cf.* Fig. 1), declining after prolonged C_2H_4 exposure. The results show that C_2H_4 causes a uncoupler, addition of 0.1 mM KCN often stimulated O_2 consumption (data not shown), an observation also noted by Lange (11). Presumably this is due to a Pasteur effect, increasing substrate flux to the mitochondria and stimulating respiration. Mitochondria from the CO_2-, C_2H_4-, and O_2-treated tubers were also largely CN-resistant (Table III). Treatment with CO_2, C_2H_4, and air, on the other hand, did not stimulate slice respiration and led to the development of only slight (10%) CN resistance (data not shown). It seems that CO_2, C_2H_4, and O_2 act synergistically in inducing the alternative path in potatoes.

Effect of Storage Temperature on C_2H_4-induced Respiration. Tubers are usually stored at 7 C in this laboratory, but it is necessary to transfer them to room temperature approximately 10 days prior to treatment in order to observe full C_2H_4 effects. Tubers transferred directly from the cold to a C_2H_4 and O_2 atmosphere developed very little CN resistance, even when CO_2 was present (Table IV). Mitochondria from cold-stored tubers also showed very little alternative path activity (Table IV). Intermediate lengths of time at room temperature allowed the development of some CN resistance, but 10 days were required for full alternative path evocation by C_2H_4 (Table IV). Ten days at higher

[2] Abbreviations: SHAM: salicylhydroxamic acid; FCCP: carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; TMPD: tetramethyl-*p*-phenylenediamine.

Table II. The effect of tuber storage time on ethylene-induced respiration

Tubers were harvested in mid-October and stored at 7C until 10 days prior to the experiments, when they were transferred to room temperature. On the dates shown, tubers were treated for approximately 24 hours with 10 µl/l ethylene in oxygen. Thereafter slices and mitochondria were prepared, and respiration measured as described in Materials and Methods. Succinate (10 mM) was the mitochondrial substrate, and 0.2 mM KCN was added during state 3 respiration to estimate cyanide resistance. Slice resistance was calculated after the residual rate (SHAM plus KCN-resistant) had been subtracted from the control and the KCN-inhibited rates. 1 mM SHAM and 0.1 mM KCN were used in slice experiments.

Date	Tuber respiration	Slice respiration	Slice resistance	Mitochondrial resistance
	µl CO_2/g fresh wt·hr		%	
12/11/77	27	54	100	70
1/24/78	20	40	88	55
2/6/78	19	32	54	29
2/24/78	-	47	30	-

Table III. Effect of tuber treatment with CO_2 and ethylene on slice respiration

Four month stored tubers were treated as described in Materials and Methods. 10% CO_2 and 10 µl/l C_2H_4 were used with pure O_2. O_2 consumption was measured as described in Table II. FCCP (1 uM), KCN (0.1 mM) and SHAM (1 mM) were added seriatim to the vessel, as indicated. The residual respiration (SHAM and KCN-resistant) was subtracted from both the uncoupled and the uncoupled + KCN rates to obtain the percent resistance shown in slices.

Treatment	Slice Respiration				SHAM-sensitive, CN-resistant Respiration	
	Control	FCCP	FCCP + KCN	FCCP KCN + SHAM	Slice	Mitochondrial
	µl O_2/g fresh wt·hr				%	
Experiment 1						
O_2	19	37	9	9	0	0
O_2 + C_2H_4	47	68	27	9	30	-
O_2 + CO_2	21	42	11	9	6	0
O_2 + CO_2 + C_2H_4	27	41	37	9	88	66
Experiment 2						
O_2	37	58	11	11	0	0
O_2 + C_2H_4	64	90	64	16	65	30
O_2 + CO_2	25	48	11	11	0	0
O_2 + CO_2 + C_2H_4	46	69	69	11	100	75

temperatures is the period needed for de-sweetening of tubers, the sugars being converted to starch (9).

Characteristics of CN-resistant Mitochondria. Figure 4 shows typical O_2 electrode tracings of mitochondria from tubers treated with CO_2, O_2, and C_2H_4. Succinate oxidation by mitochondria from control (CO_2 + O_2) tubers was completely inhibited by 0.2 mM KCN (Fig. 4A). By contrast, mitochondria from tubers treated with C_2H_4, CO_2, and O_2 were 70% resistant to CN with succinate or malate as substrates (Fig. 4, B and D), a value consistently obtained with mitochondria from slices 100% resistant to KCN. CN-resistant mitochondria were also antimycin-resistant (data not shown). Exogenous NADH oxidation was considerably more sensitive to CN (Fig. 4C), as observed previously (7, 21, 29). Despite participation of the CN-resistant path, C_2H_4-mitochondria remain coupled (Fig. 4D and Table V). Inasmuch as the alternative path *per se* is nonphosphorylating (2, 23), the degree of coupling to phosphorylation will depend on the extent to which the alternative path is engaged during state 3, and on the capacity of the alternative path (2). The inhibition of succinate oxidation by SHAM in the absence of CN indicates that the alternative path contributes significantly to the total respiration, and the high ADP/O values obtained in the presence of SHAM indicate that the Cyt chain continues to phosphorylate efficiently (Table V). Malate oxidation is more extensively coupled to ATP formation (Fig. 4D) because the first phosphorylation site remains operative during alternative path activity. In addition to being resistant to CN, mitochondria from C_2H_4-treated tubers display higher intrinsic oxidation rates than those from control tubers, except when ascorbate + TMPD are substrates (Fig. 3 and Table VI). This result was obtained consistently with both washed and purified mitochondria (Table VI) although the difference in rates varied somewhat (compare Table VI with Fig. 3). The response to C_2H_4

Table IV. The effect of storage temperature on ethylene-induced respiration

Tubers were treated as described in Materials and Methods. Assay conditions are described in Table II. Tubers in experiment A were approximately two months from harvest. Tubers in experiment B were approximately 4 months from harvest. Cyanide resistance calculated as in Tables II and III.

Treatment	Storage temperature	Slice respiration		Cyanide resistance
		Control	+0.1 mM KCN	
		μl O_2/g fresh wt·hr		%
A. 24 hours in O_2	7 C	48	8	0
+ C_2H_4	7 C	67	10	0
	6 days at room temp.	50	25	38
	10 days at room temp.	39	38	97
B. 24 hours in O_2	7 C	58	24	29
+ C_2H_4 + CO_2	10 days at room temp.	55	55	100

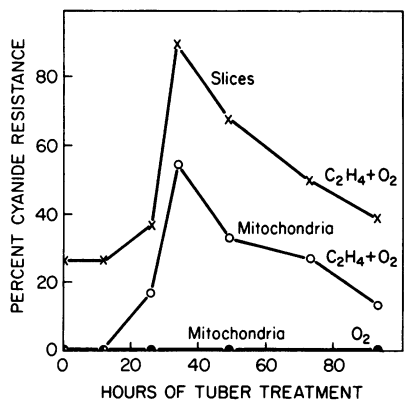

FIG. 2. Effect of C_2H_4 tuber treatment on CN resistance in potato slices and mitochondria. O_2 uptake was measured polarographically as described under "Materials and Methods." Succinate (10 mM) was the mitochondrial substrate, and 0.2 mM KCN was added during state 3. Mitochondrial CN-resistant O_2 uptake was completely inhibited by 2 mM SHAM. Tubers were treated with C_2H_4 + O_2 as described in Figure 1, and slices and mitochondria were prepared from sample tubers at the times indicated. (O——O): mitochondria from C_2H_4 + O_2-treated tubers; (●——●): mitochondria from control (O_2-treated) tubers; (×——×): slices from C_2H_4 + O_2-treated tubers.

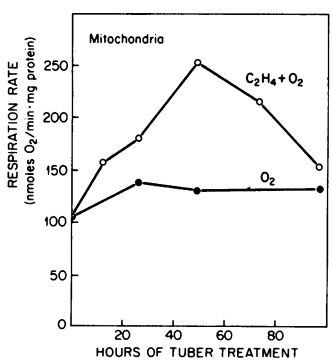

FIG. 3. Effect of C_2H_4 tuber treatment on succinate oxidation by potato mitochondria. Experimental details the same as in Figure 3. State 3 rates of O_2 consumption are shown. (O——O): mitochondria from C_2H_4-treated tubers; (●——●): mitochondria from control tubers.

was more pronounced with malate than with other substrates (Table VI). The results with ascorbate + TMPD show that Cyt oxidase activity is not affected by C_2H_4 treatment. The enhanced

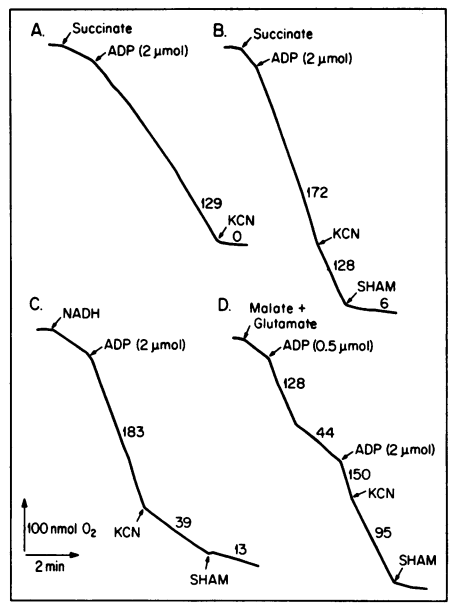

FIG. 4. O_2 consumption by mitochondria from tubers treated with C_2H_4 + CO_2 and O_2. O_2 uptake was measured as described under "Materials and Methods," with 10 mM succinate, 1 mM NADH, or 10 mM malate as substrate. When malate was used, 10 mM glutamate was included in the standard reaction mixture. ADP, KCN (0.2 mM), and SHAM (2 mM) were added as indicated. A: mitochondria from tubers treated with 10% CO_2 in O_2, for 24 hr; B, C, and D: mitochondria from tubers treated with C_2H_4 + CO_2 and O_2. Rates are expressed as nmol of O_2/min·mg of protein.

mitochondrial respiratory rates are dependent primarily on C_2H_4. Omission of CO_2 from the gas mixture with which tubers were treated results in less CN resistance but a similar stimulation of substrate oxidation (Table VI).

DISCUSSION

The results presented here confirm those of Rychter and colleagues (21, 22) and show that C_2H_4 can induce alternative path development in potatoes. CO_2 and O_2 augment the process. CO_2 becomes necessary only after lengthy storage of the tubers, suggesting that in younger tubers CO_2 accumulation upon treatment with C_2H_4 and O_2 is great enough to cause alternative path development without the need for added CO_2. The tubers become noticeably soft with long periods of storage, and it may be that permeability to gases increases, leading to lower intracellular CO_2

Table V. Energy conservation by mitochondria from tubers treated with ethylene, oxygen and CO_2.

Assay conditions with succinate as substrate are described in Table II. 2 mM SHAM was used.

Experiment	Treatment	State 3 respiration	State 4 respiration	R.C.R.	ADP/O
		nmoles O_2/min.mg protein			
1	Control	178	122	1.45	1.3
	SHAM	122	77	1.60	1.8
2	Control	205	150	1.40	1.2
	SHAM	159	91	1.75	1.4

Table VI. The effect of tuber treatment with ethylene on the specific activities of washed and purified mitochondria

Oxygen consumption was measured as described in Materials and Methods. Rates shown are state 3 rates obtained with 10 mM succinate, 10 mM malate + 10 mM glutamate, 1 mM NADH, or 10 mM ascorbate + 5 mM TMPD. 0.2 mM KCN was used where indicated. Tubers were used approximately 5 months from harvest. Control tubers treated with pure oxygen. CO_2 was 10%.

Substrate	CONTROL			ETHYLENE + O_2		
	Washed	Purified		Washed	Purified	
	State 3	State 3	+KCN	State 3	State 3	+KCN
	nmoles O_2/min. mg protein					
Succinate	100	153	0	172	222	78
NADH	95	100	0	172	156	-
Malate + Glutamate	48	50	0	117	106	36
Ascorbate + TMPD	-	443	0	-	443	0

	CONTROL			ETHYLENE + O_2 + CO_2		
	Washed	Purified		Washed	Purified	
	State 3	State 3	+KCN	State 3	State 3	+KCN
	nmoles O_2/min. mg protein					
Succinate	111	130	0	137	192	125
NADH	117	125		175	183	40
Malate + Glutamate	56	61	0	156	130	90
Ascorbate + TMPD	-	567	0	-	583	0

levels. The slight decrease in CO_2 evolution by the tubers with age (Table II) may also contribute to the need for added CO_2. Recent work with artichokes and potatoes has shown that changes in membrane properties take place during storage of tubers (Wright and Raison, personal communication), lending support to the above hypothesis. However, other metabolic changes occur and may influence C_2H_4 responses. With prolonged storage tubers begin to sprout, and changes in sugar levels also occur (8). Whatever the reason, it is obvious that C_2H_4, CO_2, and O_2 act synergistically to induce CN resistance in potatoes. Development of the alternative path in etiolated wheat coleoptiles upon CO_2 treatment has been observed (19, *cf.* 11). Although a definite role for C_2H_4 was not shown, endogenous C_2H_4 production was not eliminated as a possibility, and may have been contributory. Synergism between CO_2 and C_2H_4 has been observed in dormancy release in thermodormant lettuce seeds (10) and in the activation of pea cotyledon ATPase (17).

Mapson and Burton (18) described two operative terminal oxidases in potato tubers—one with a high affinity for O_2, taken to be Cyt oxidase, and one with an affinity at least 2,000 times lower. The O_2 affinity of the SHAM-sensitive, CN-resistant oxidase, on the other hand, has been estimated to be but 10 times lower than that of Cyt oxidase (23). Whereas the low affinity oxidase described by Mapson and Burton *may* be involved in the C_2H_4 response, there is no difference in the respiration rate of C_2H_4-treated tubers in air and in O_2 in the first 8 to 10 hr, during which time the respiration rise is nominal (Fig. 1, *cf.* 5). Since in bulky tissues O_2 becomes limiting even to Cyt oxidase-mediated respiration at elevated respiration rates (see ref. 13), the high O_2 requirement noted herein, and by Rychter and Frenkel (21), may be to sustain rather than to elicit high respiration rates.

The observation that C_2H_4 induces CN resistance led Rychter *et al.* (22) to suggest that the alternative path is absent in dormant tubers, and develops when C_2H_4 is provided. Previous work from

this laboratory (15, 27, 30) has suggested that the alternative path is present in tubers but is labile and lost upon slicing, due to lipid breakdown. The development of CN resistance in aged potato slices requires phospholipid synthesis (30), suggesting that phospholipid, and, presumably, membrane, integrity is essential for the operation of the alternative path. Inasmuch as the result of tuber incubation in C_2H_4 and CO_2 may be to prevent lipid breakdown during slicing, and hence to preserve CN resistance, it has not been possible to decide between the two hypotheses on the data provided. CN-resistant fresh slices from C_2H_4-treated tubers continue to resemble ordinary fresh slices in being unable to oxidize exogenously provided glucose (Christoffersen, unpublished). Ordinary *aged* slices, which are also CN-resistant, oxidize exogenous glucose handily (13). Further, CN-resistant C_2H_4 slices are as sensitive to antimycin as to CN in the presence of SHAM, in contrast to normal CN-resistant aged slices which are relatively insensitive to antimycin in the presence of SHAM (28).

The results also show that C_2H_4 stimulates the respiration *per se* of tubers, slices, and mitochondria (the increase in slice respiration is apparent even when uncoupler is present—Table III). Respiratory stimulation seems to be independent of CO_2 administration and alternative path development, since C_2H_4 and O_2 alone stimulate O_2 uptake in old tubers, which develop little resistance to CN in the absence of CO_2 (Tables III and VI). The increase in uncoupled (*i.e.* maximum) slice respiration and mitochondrial O_2 uptake suggests that C_2H_4 causes some development of respiratory capacity rather than simply unmasking a latent capacity of the dormant tuber. Slice respiration is lowered when CO_2 is added with C_2H_4 (Table III), whereas mitochondrial respiratory rates are very similar (Table VI). This implies that high CO_2 concentrations repress substrate flux to the mitochondria. Inhibition of slice respiration by CO_2 has been noted previously (14, 18), but the mechanism of inhibition remains unclear.

How C_2H_4 stimulates CN-resistant and CN-sensitive respiration is not clear, but it is unlikely that an immediate direct effect on the mitochondria is involved, since administration of C_2H_4 to isolated sweet potato mitchondria has no detectable effect on their respiratory chains (1). Likewise, the decrease in respiratory rates after 40 hr of C_2H_4 treatment (Figs. 1, 2, and 3) is not consistent with an immediate direct effect of the gas on the mitochondria. The stimulation of mitochondrial respiration may involve activation of respiratory dehydrogenases. In cauliflower mitochondria it is this part of the respiratory chain that restricts electron flow with any one substrate (6). In potato mitochondria the different degrees of stimulation observed with different substrates following tuber treatment with ethylene (Table VI) support the view that dehydrogenase activation may be at issue. Consistent with this suggestion is the absence of any change in Cyt oxidase activity. In the presence of SHAM, the specific respiration rate is greater in mitochondria from tubers treated with C_2H_4 and O_2 whether or not CO_2 is present (data not shown). The increase in intrinsic mitochondrial respiratory activity in C_2H_4-treated tubers does not depend on the development or manifestation of the alternative path.

Finally, the results support previous implications of alternative path involvement in other C_2H_4 responses (26).

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